Demonstration of Ozone Impacts on Crop Species in the San Joaquin Valley:

Open Top Chambers at Kearney Agricultural Center

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ABSTRACT

The San Joaquin Valley exceeds state and federal air quality standards for ozone. Agricultural interests are politically and socially important, yet despite documented crop damage and yield loss due to ozone, this segment of the population has been less supportive of air quality regulatory activity than others. A research and demonstration project was designed to increase recognition of ozone symptoms in crops of the ozone problem in general. The object was to increase participation in air quality improvement initiatives. Plants were exposed in Open Top Chambers and a web site was developed for non-specialist and specialist adults, and for children. Visits, site tours and other activities were hosted at the University of California Kearney Agricultural Center in Parlier. Research activity provided demonstration materials. Outreach was limited during the period of the project as economic pressures cancelled many tours and air quality regulatory issues contentious. Useful outreach materials and a continuing physical and web-based presence were established. As this program was concluding, a similar program for children, Orchard Odyssey, is under development, using materials and initiatives of this program. Future air quality demonstration should be housed in a greenhouse to allow year round demonstration, and should continue to incorporate a research component.

EXECUTIVE SUMMARY

Background

The San Joaquin Valley (SJV) is a large inland airbasin, largely agricultural but increasingly urban, that currently exceeds state and federal air quality standards for ozone. Due to increasing population, industry, and vehicle miles traveled, it is unlikely that the SJV can meet air quality standards in the near future. In June 2000, the SJV was downgraded from Serious to Severe Non-Attainment. At the present time, it is under consideration to voluntarily further downgrade from Severe to Extreme Non-Attainment. Agriculture is a politically and socially important sector of SJV society. Despite documented crop damage and yield loss due to ozone, this sector has been less supportive of regulatory activity than other, more urban, sectors. The reasons for this are complex, but include a lack of adequate familiarity with the issues surrounding crop loss and human health impacts of ozone pollution. The objective of the project was to increase such awareness among both adults and children in an agricultural region, in order to increase "buy-in" and participation in air quality improvement initiatives. A combined research and demonstration project was designed to increase recognition of ozone symptoms and awareness of the ozone problem among this segment of the SJV population.

Methods

A variety of locally important crop plants, and visually interesting ornamental species was exposed to three ozone concentration regimes in Open Top Chambers (OTCs). Three replicate chambers were exposed to each of three ozone concentrations: charcoal filtered air (CF; nominally 12 hour mean, 12 hm, = 0 ppb), Moderate Ozone (MO3; 12 hm = 90 ppb) and High Ozone (HO3; 12 hm = 143 ppb). MO3 was designed to follow the diurnal dynamics and magnitude of local ozone concentrations on notably polluted days. HO3 tracked at 1.6 times MO3. Direct demonstration, through tours and site visits, with symptomatic plants, was combined with research data derived from the same plants, and with a comprehensive web site containing data, publications and links to other air quality sites. This multi-phasic approach was designed to provide a broad based educational experience for interested visitors. Information and activities were designed for both non-specialist and specialist adults, and for school age children. Materials were also assembled for school teachers, to facilitate their further incorporation of the demonstration materials into ongoing curricula. All physical and web-based activities were hosted at the University of California Kearney Agricultural Center (KAC) in Parlier. This was designed to maximize contact with growers, packers, and public and private sector farm advisors, including University of California Cooperative Extension Advisors and Specialists. Additionally, the Kearney Research and Extension Center (KREC) hosts a large number of scientific and extension meetings, as well as a generally well-attended high school outreach program, AgFutures. This latter program which draws interested students from around the SJV. Ongoing tours of the KREC facility have incorporated earlier air quality installations. This purpose built facility was designed to enhance the outreach that was already occurring, and to increase and diversify the number of visitors.

Results

Pima cotton and muskmelon (cantaloupe) were the most successful demonstration species, even when grown in near-hydroponic conditions in pots of sintered clay. Kiwifruit grew well but exhibited no visible symptoms despite demonstrated physiological impacts of ozone. Almond, grape, and peach, important local horticultural crops, did not perform well in pots over extended periods in the OTCs, even when grown in high quality potting media. Off season (winter) cultivation of sweet peas was attempted, but was unsuccessful due to low available light in this foggy environment. Outreach was of only limited effectiveness during the difficult economic conditions of the project period. Visitor numbers were markedly reduced at KREC, and the AgFutures program was cancelled for the first time. The contentious state of air quality regulatory activities with respect to agricultural interests also reduced interest among the target population. The research on mechanisms of ozone injury to key crops provided interesting demonstration materials, as well as material for public and scientific presentations. The availability of such information generated interest from the media and from local academic institutions. Several media tours were conducted, and a few news articles appeared, along with many other opportunities to provide background information for investigative reports. This increased the effectiveness of local reporting on air quality issues during a period of intense media scrutiny of air quality regulatory activity in the SJV. This intensity contributed both to the lack of agricultural participation in the program, as attitudes hardened and the population polarized and contributed to interest in the project. Research with cotton and melon plants, both grown extensively in the SJV, yielded quantitative characterization of root system inhibition by exposure of the plant shoot to ozone. Results indicated that root respiration and oxygen consumption increased with ozone exposure. This suggests that a damaging signal is transferred from shoot to root, because the roots themselves are not exposed to ozone. Results also showed that the restriction of root development is largely confined to the very finest roots, which is particularly damaging since these roots absorb water and nutrients. Kiwifruit leaves did not exhibit visible symptoms of discoloration, stippling, nor bronzing, at any concentration of ozone. Overall health of the plants, however, was only moderate, with considerable marginal leaf necrosis in all ozone treatments. Photosynthesis was inhibited by ozone. Kiwifruit stems, which produce abundant undesirable basal sucker development, did not do so after exposure to ozone. There was an indication of greater suckering from above the graft union in the higher ozone treatment than in lower ozone concentrations. A more rapid leaf turnover and consequent reduced leaf life span was observed in the high ozone treatment, and was suggested in the moderate ozone treatment. Stomatal conductance declined with increasing ozone concentration, as expected. However, photosynthetic carbon assimilation declined from CF to MO3, but then increased with further increase to HO3. This latter observation is anomalous, and reflects inadvertent assay of younger leaves in HO3 than in the other treatments, due to the greater turnover of leaves noted above. Roots in all treatments appeared healthy, though ozone impacts on root development could not be ruled out.

Conclusions

Useful outreach materials and a continuing physical and web-based presence were established. Substantial research results were obtained. As the current CARB-supported program concludes, another program for local school children, Orchard Odyssey, is being revived and further developed. This program will carry forward many of the initiatives and utilize many of the resources created by this program. It will continue to feature air quality as a major focus of student activities. The air quality demonstration components will be housed in a new research greenhouse at KREC to allow for year round demonstration, meeting a significant limitation of the current program. Future programs of this type should continue to incorporate a research agenda as well as a demonstration and outreach component. Both missions should be adequately supported to insure success. The continuing existence of such programs in both urban and rural environments is desirable, making a potentially useful contribution to emerging political consensus on air quality regulation.

INTRODUCTION

The San Joaquin Valley (SJV) of California is a large inland airbasin, largely agricultural but increasingly urban, that currently exceeds state and federal air quality standards for ozone. The SJV is one of the three most polluted air basins in the U.S., along with Los Angeles and Houston. Parlier, the site of Kearney Agricultural Center (KAC) and the Kearney Research and Extension Center (KREC) at which KAC is located, is often the most polluted area of the SJV. During the May through October period, daily maximum ozone concentration typically exceeds 0.90 parts per billion by volume (ppb), the California state regulatory standard based on human health (Fig. 2). These short term ozone exposures, as well as the 8 hour average exposures considered more indicative of biological damage, are well above levels known to harm sensitive vegetation.

Ozone is only a moderately reactive gaseous compound. But tropospheric ozone is ubiquitous, and poses the greatest threat to vegetation of any air pollutant (Krupa and Kickert, 1989; Krupa and Manning, 1988), to natural and agricultural ecosystems throughout the United States and the world. Damage to crop plants in the United States may exceed several billion dollars per annum, depending on assumptions regarding price elasticity (e.g. Heck et al., 1983; Adams et al., 1988). Ozone does not enter the soil and hence acts exclusively on the shoot, yet ozone exposure substantially alters root system development in many plants (Cooley and Manning, 1987). The role of root systems in the integrated function of whole plants, including substantial control of shoot functions such as gas exchange and growth, has come under increasing study in recent years (Meinzer and Grantz, 1990; Passioura, 1988; Zhang and Davies, 1989). This approach considers direct and indirect effects on shoot water relations and photosynthetic gas exchange, potentially mediated by materials transported from root to shoot in the transpiration stream (Meinzer and Grantz, 1991).

In general, wild plant species subjected to adaptive pressure in polluted environments develop increased resistance to ozone over time. This is also true of crops such as cotton that have been selected for yield under the high ozone conditions of the San Joaquin Valley (Grantz and McCool, 1993). Newly introduced crops, such as the long staple Pima cottons and kiwifruit typically exhibit markedly greater sensitivity to ozone than locally adapted cultivars.

Due to increasing population, industry, and vehicle miles traveled, it is considered unlikely that the SJV can meet air quality standards in the near future. In June 2000, the SJV was downgraded from a Serious to a Severe Non-Attainment Area. At the present time, it is under consideration to voluntarily further downgrade the SJV from Severe to Extreme Non-Attainment. Rising tropospheric ozone is also an important component of Global Change that, alone and in concert with other factors such as water availability and temperature, may impact the sustainability of currently productive agricultural and natural biological systems.

Due to the pernicious and increasing impacts of tropospheric ozone on human health and welfare, greater societal awareness of the problem is desirable. Agriculture is a politically and socially important sector of SJV society. Despite documented crop damage and yield loss due to ozone, this sector has been less supportive of regulatory activity than other, more urban, sectors. The reasons for this are complex, but include a lack of adequate familiarity with the issues surrounding crop loss and human health impacts of ozone pollution.

A combined research and demonstration project was designed to increase recognition of ozone symptoms and awareness of the ozone problem among this segment of the SJV population. The demonstration materials were taken from ongoing research activities. These were focused on the hypothesis that ozone damages crops by inhibiting carbohydrate transport to developing roots. This is suggested to cause direct and indirect impacts on physiological processes and on yield. Characterization of limitations to root development, with consequent reduced yield capacity of the shoot, suggests novel approaches to improvement of air pollution resistance in annual crop plants and in perennial plants such as forest tree species. The basic and applied information to be derived from a whole plant evaluation of ozone impacts also contributes information that can minimize the disruptive consequences of Global Change as it occurs due, in part, to rising tropospheric ozone.

This project used two contrasting plant species, Pima cotton (*Gossypium barbadense* L.), which translocates sucrose, and muskmelon (canteloupe, *Cucumis melo* L.), which translocates stachyose to investigate and demonstrate ozone impacts on carbohydrate movement. Both Pima cotton and muskmelon are of considerable economic importance in many areas of the United States, the SJV, and throughout the world. Both are biologically representative of large classes of plants.

Pima cotton has recently been introduced into the San Joaquin Valley (SJV) of California, where yields and quality have been very high relative to other production areas. Advanced cultivars were originally selected under low ozone pressure in Arizona, and have proven to be sensitive to ozone under more typical SJV conditions. Field exposure chamber studies indicated that Pima cv. S-6 was considerably more sensitive to ozone, in fiber yield and quality, than the widely cultivated upland cotton (*Gossypium hirsutum* L.) cv. SJ-2 (Grantz and McCool, 1993). Field observations (Olszyk et al., 1993) also suggested a high level of sensitivity of S-6 to ozone, across a natural gradient of ozone concentrations in the SJV. Increased stomatal conductance in advanced Pima lines (Cornish et al., 1991) may contribute to the high sensitivity of S-6 to ozone. Reduction of carbon allocation to roots following exposure to ozone is observed in upland cotton (e.g. Oshima et al., 1979), and for Pima cottons (Grantz and Yang, 1996), as well as many other plant species (Cooley and Manning, 1987).

Muskmelon and other cucurbits represent another important horticultural crop in the United States and elsewhere in the world. Muskmelon utilizes the raffinose oligosaccharide, stachyose, as the major phloem transport compound (Mitchell et al., 1992). Many horticulturally important plants are stachyose transporters, including many herbs, other cucurbits, avocado trees and many ornamentals (e.g. Bachmann et al., 1994). The response of muskmelon to ozone is very interesting (Fernandez-Bayon et al., 1993; Snyder et al., 1988; Gausman et al., 1978). Carbon assimilation is impacted relatively rapidly, along with effects on chlorophyll <u>a</u> fluorescence, chlorophyll content, stomatal conductance, and leaf visual symptoms. The number of flowers and fruit per plant is reduced by chronic ozone exposure which leads to substantial yield reductions. However, the relative growth rate of both roots and shoots are maintained.

In a comparison of broad beans (*Vicia faba* L.), a sucrose transporter, with basil (*Ocimum basilicum* L.), a stachyose transporter, effects of ozone and sulfhydryl reagents on rates of phloem exudation were restricted to the bean. Basil did not exhibit responses of phloem exudation to ozone exposure (M. Madore, personal communication). Differences in response to ozone thus may be of practical horticultural significance, as well as providing a useful model system to help unravel the mechanism of ozone effects on whole plants. Phloem transport has frequently been suggested as a possible site of ozone action (e.g. Mortensen and Engvild, 1995), but this has never been demonstrated. Use of these contrasting systems provides an interesting range of demonstration opportunities.

The objective of the project was to increase awareness of ozone air pollution among both adults and children in an agricultural region, in order to increase "buy-in" and participation in air quality improvement initiatives. The visual and physiological diversity of the plant materials chosen were intended to provide a broadly interesting selection of ozone impacts. The species selected also had a scientific basis, directed toward answering mechanistic questions to help predict ozone damage to native and agricultural vegetation.

MATERIALS AND METHODS

Preparations began in Spring 2001 for experimental exposure of plants. A site of approximately one-half acre was obtained from the Kearney Research and Extension Center located in Parlier, California, southern Fresno County (Figure 1).

Figure 1. Kearney Agricultural Center is a rich and diverse research and extension environment with advanced laboratories and a large number of field and horticultural crops.

The site is surrounded by a research orchard of deciduous fruit trees to the east and south with the pavilion to the west and a research field of alfalfa to the north. The site is accessible to buses and to the tram operated for visitors by the Kearney Research and Extension Center. The site was leveled, appropriate drainage was constructed and hydraulic separation erected between site and neighboring alfalfa fields. The site is located in the center of the Kearney Research and Extension Center research farm adjacent to a previously constructed outdoor pavilion providing space for educational programs involving school-age children.

A 40-foot mobile laboratory was converted from a mobile office building. The mobile laboratory was leased from a commercial vendor. The laboratory was divided into three rooms. One room was devoted to the computer equipment and hardware involved with dosing and measurement of ozone in the open-top chambers (OTCs). The middle room in the mobile laboratory contained digital scanners to image root systems, a computerized system for geometric analysis of these root systems, and a computerized system for measuring the respiration of isolated roots from the potted plants. The third room contained a sink for washing the sintered clay from the roots to allow these measurements of root respiration and root morphology. This room also contained calibration gases and materials to maintain the portable photosynthetic gas exchange measurement system that was used *in situ* in the OTCs.

Both the second and third rooms provided space to erect educational displays such as posters and to stage demonstrations of ozone effects on plants. Physical science demonstrations of ozone effects on materials such as rubber bands, were also provided, of discoloration of gases (smog formation) in a partially evacuated laboratory flask.

Ten open-top chambers were erected on the site in December 2001 (Figure 2), including the aluminum framework and plastic sheeting. This was a change from the original design of 8 OTCs. This has allowed us to replicate 3 concentrations of ozone, 3 times for increased statistical rigor, and also provided a spare OTC for other applications or for redundancy. The chambers are of the 3 meter (ten-foot) diameter, circular, NCLAN design. They include upper sections of conical frustra but do not include rain shelters. Rain is not considered likely during the growing season in the San Joaquin Valley. The plastic sheeting initially installed was retained from a previous project. The light transmitting properties were sufficiently degraded that these plastic covers were replaced during Spring 2002.

The floor of each chamber was covered with an opaque water- and gas-permeable plastic weed matting to suppress the growth of weeds. This material has been covered with pea gravel to provide a more durable surface on which to place the pots.

Each chamber was washed weekly with running water and a soft brush, to remove dust and pollution collecting on the exterior of the plastic cover.

By Fall 2001 the OTC site was functional though further tuning of the ozonation system was required. The system was fully operational and was utilized to expose plants to ozone during the growing seasons of 2002 and 2003.

Figure 2. Ozone fumigation experiments at Kearney Agricultural Center were performed using computer controlled Open Top Chambers (OTCs). In this case kiwifruit vines were subjected to high ozone concentrations.

Ozone Exposure

Ozone was generated from oxygen, rather than air, to avoid phytotoxicity problems arising from generation of N_2O_5. An oxygen concentration system G-22; Pacific Ozone Technolgies was installed to provide nearly pure (>99%) oxygen to the ozone generator. Original plans to use an older Griffin ozone generator, were altered in favor of using this much smaller and more energy efficient unit. The G-22 relies on corona discharge at lower voltage (about 5 kV) than the older unit. This required some changes in the data acquisition system, including shielding and appropriate electrical grounding, to overcome the radiation noise generated by the new ozone generator.

The ozone generator was computer-controlled through a feed back system, to control the ozone concentrations in each of the open-top chambers. The ozonation was initially not as precise with this system as has been achieved with other systems. The problem was isolated to a poorly designed voltage control sensor provided as original equipment in the ozone generator. During Fall 2001 the problem was addressed by altering the algorithm in the control software, to include over-sampling and averaging of the feedback signal. In this way an excellent level of ozone control was achieved.

This system with modifications represents a significant advance over previous designs of OTC facilities.

The exposure protocol developed at the University of California Kearney Agricultural Center (KAC), was based on the most highly polluted days over several recent years of observation. The daily progression of ozone concentrations was parameterized as a series of step-wise linear functions. This timecourse, and the magnitude of hourly values, was maintained constant from day to day. The three ozone exposure regimes were characterized as 12 hour means (12 hm) over the daylight period. These average values were nominally 0, 90, and 143 ppb (Grantz et al., 2003). The lowest concentration was Charcoal Filtered air (Fig. 3, open circles). These chambers (CF) were exposed under operating conditions to 12 hour mean, (12 hm) = 16 ppb). The non-zero value is due to filter imperfections and introgression of ambient air against the bulk flow of air through the open top of the chamber.

The middle, near-ambient, concentration was designed to be somewhat below the mean concentration of the most polluted days of three recent years of ambient observations (Fig. 3, shaded circles). As ozone concentrations tend to exhibit peaks and valleys of several days duration throughout the season, this maximum exposure day after day represents a greater imposition of ozone than in the local ambient environment. Its consistency, while maintaining an environmentally relevant range, is conducive to reproducible measurements. This Moderate Ozone (MO3; 12 hm = 81 ppb) treatment was designed to follow the diurnal dynamics and magnitude of local ozone concentrations on notably polluted days.

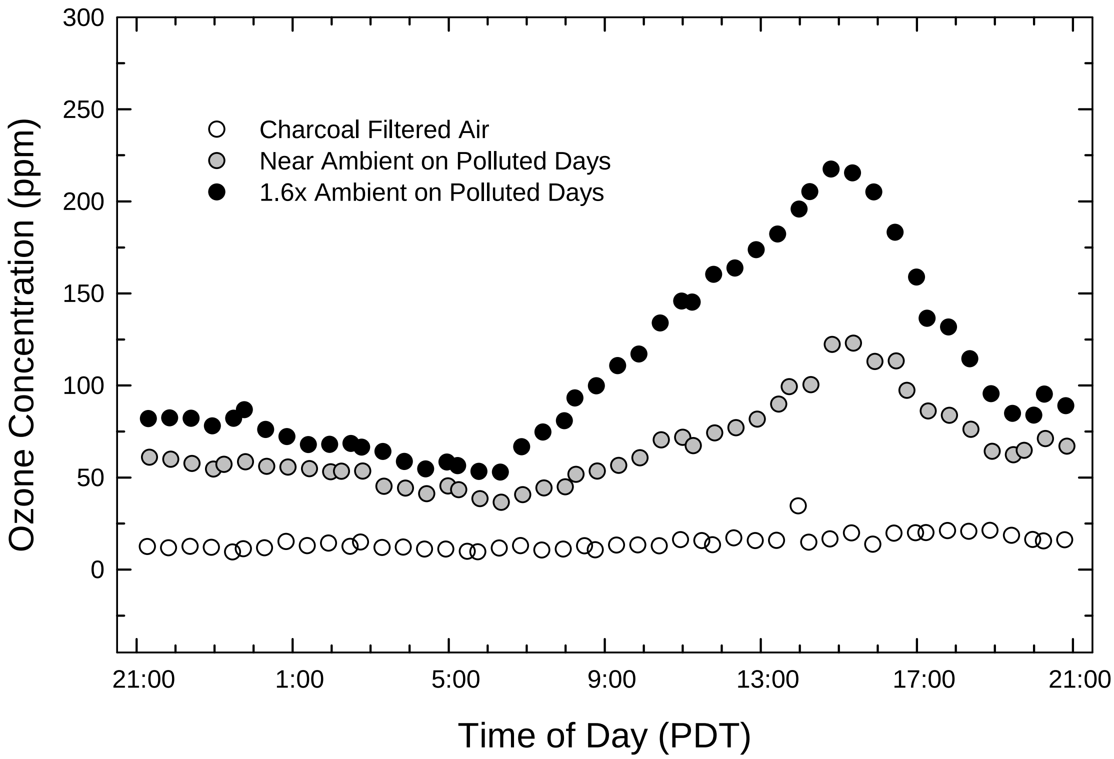
The High Ozone (HO3) concentration (Fig. 3, solid circles) tracked at 1.9 times that of the near-ambient (MO3) treatment. The HO3 OTCs were exposed to 12 hm = 154 ppb. Even the midafternoon peak concentration in this treatment was not environmentally unreasonable. Concentrations much greater than this were observed in the Los Angeles airbasin until recently, and are still occasionally observed there and in some developing areas.

Ozone was sampled in each chamber through Teflon tubing using a computer controlled multi-port solenoid sampling valve (Scanivalve). All concentrations were analyzed with a single ultraviolet absorption monitor (ThermoElectron 49C). A second monitor was used to sample a single chamber (the Master Control Chamber) on a continuous basis. All feedback adjustments were made based on the Master Chamber. The analog output signal for the 49C monitor was used to regulate the voltage of the G-22 generator. Both monitors were calibrated using a secondary standard that was calibrated annually at the CARB laboratory in Sacramento.

Ozone concentrations in the chambers other than the Master Chamber were adjusted proportionally to the Master Chamber, using manual flow meters (rotameters). Flow of high concentration ozone from the G-22 to each chamber was initially adjusted to a predetermined fraction of the flow to the Master Chamber. Fine adjustment of the flow meter for each chamber was performed on a continuing basis throughout the project, using monitored ozone concentrations in each chamber.

During the 2003 growing season the ozone exposure profile provided excellent control. The actual concentration achieved, in the CF treatment, about 12 hm = 16 ppb, were nearly ideal for reference similar to pristine ambient air.

Fig. 3. Representative daily timecourse of ozone concentrations in Open Top Chambers at Kearney Agricultural Center.



Establishment and maintenance of vegetation for research and demonstration

A drip irrigation and automated fertilization systems was installed “Fertigation” was applied automatically on the basis of a timer to each pot in each of the 9 chambers. Following some initial experimentation with various irrigation and fertigation scheduling, plants were fertilized through the irrigation system 3 times weekly using premixed *Miracle Grow* fertilizer, and irrigated with water twice daily. To decrease root stress and reduce root temperature caused by the black plastic pots or sleeves, reflective bubble wrap insulation was secured to the outside of each pot.

A variety of locally important crop plants, and visually interesting ornamental species was exposed to the three ozone concentration regimes in OTCs. This included pistachio, grape, peach, almond, kiwifruit, and several ornamental houseplant species obtained from local nurseries, as well as pima cotton and muskmelon (cantaloupe). Cotton and melon were maintained for demonstration, through frequent planting, because the symptoms were consistent and showed well to agriculturists. The ornamental plants, notably carnations, roses, and petunias, were maintained because their blooms were more appealing to the eye, foliage and plant size reflected ozone exposure, and visitors from the non-agricultural sector were more familiar with these species. The ornamental species required replacement with new nursery material frequently during the season.

During Spring 2001 the growth conditions for the demonstration plants were determined. Deep pots were desirable for cotton, which has a tap root system. Total pot volume was more important for melon plants, which have a fibrous root system. These competing requirements, while maintaining a pot size that could be easily manipulated by one person, led to selection of 1.6 ℓ (four-gallon) "tall boy" (45 cm) pots, approximately 18 cm (7 inches) in diameter. Plants were seeded directly into the pots, and germinated and grown in the OTCs, subjected to contrasting ozone exposures.

The software tools associated with root respiration and root morphology instrumentation was installed and preliminary testing began during Fall 2001.

A variety of custom and commercial potting materials was investigated for potted plant growth in the OTCs. Varying proportions of sawdust and decomposing bark produced excellent plant growth, however, all organic material including the peat moss in most potting mixes led to unacceptable levels of adherence to the fine roots. Large particles of wood, bark, or soil adhering to the roots made it difficult to obtain valid dry weights of root system components, and also confused the digital analysis system used to characterize root morphology. This was a minor problem in Pima cotton but was a major problem with the fibrous root system of muskmelon. A local source of sintered clay was located and a truck load of the material (6-4D mesh) was ordered and delivered to the site.

Excellent growth of both cotton and melon was achieved. Though the material is relatively devoid of nutrients, it is friable and easily removed from the fine roots. Nutrients are supplied through the fertigation system.

Over the winters of 2001 and 2002, plant material was maintained in the chambers in the off-season. This included the locally important perennial species pistachio, grape, peach, and almond, plus a twice weekly planting of annual sweet peas. It was intended that sweet peas could grow in the foggy and cold winter season in the SJV to provide plant material for off season testing of physiological monitoring equipment and to allow year round demonstration and research. However, overwintering sweet pea plants did not thrive nor did the perennials maintained over extended periods in the OTCs. Several of the ornamental plants (particularly carnations and petunias) that had developed under the contrasting ozone exposures at the end of the previous summer growing season were kept in the chambers during the early part of the fall and winter. They did not continue to grow, but maintained their flowers, and visually reflected the ozone-induced differences in growth.

Exceptionally cool weather during Spring of 2003 delayed the successful cultivation of cotton, melon and the ornamental species. In particular, seed germination and seedling emergence were weak. By June, however, the growing season weather had become ideal for these plant species.

Tomato and a common local weed species, yellow nutsedge, were added to the chambers in early summer of 2003. These were also grown in sintered clay to facilitate measurements of the crop-weed interaction in the soil.

Kiwifruit plants were placed in the chambers in July 2003. They were obtained from Brokaw Nursery Inc., Saticoy, CA. The plants were in 3 gallon black plastic sleeve pots with open tops and bottoms. Each pot was filled with an organic potting soil mix from the nursery. The plants were approximately one year old when received, with the roots already filling the pots. Four plants were placed in each of 3 OTCs exposing each group of plants to a different concentration of ozone. Plants were not repotted, and thus were not grown in the sintered clay media, unlike the other experimental plants.

After the kiwifruit plants had been exposed to ozone for 2 weeks, measurements of photosynthetic gas exchange were made with a steady state gas exchange system (LiCor 6400). Conductance of top and bottom leaf surface stomatal conductance and leaf surface temperatures were collected with a single sided porometer (LiCor 1600).

Sucker shoots were trimmed from the plants, also at 2 weeks after initiation of exposure in June. In Fall (October 15, 2003) suckers were collected from main stems of all plants. Suckers originating below the graft union, were separated from those originating from above the graft. Suckers were dried at 60C to constant weight and weighed after being collected.

At the conclusion of the 2003 growing season, the plants were severed at the soil line. The roots were washed to remove as much potting medium as possible, photographed and dried. Dry biomass was recorded.

Development of outreach programs

Tours and site visits provided direct visitor contact with the symptomatic plants grown in OTCs. This was combined with research data derived from the plants, and with a comprehensive web site, to provided a multi-phasic approach. This was designed to provide a broad based educational experience for interested visitors. Information and activities were designed for both non-specialist and specialist adults, and for school age children. Materials were also assembled for school teachers, to facilitate their further incorporation of the demonstration materials into ongoing curricula.

All physical and web-based activities were hosted at the University of California Kearney Research and Extension Center (KREC) in Parlier. This was intended to maximize contact with growers, packers, and public and private sector farm consultants including University of California Cooperative Extension Advisors and Specialists. KREC hosts a large number of scientific and extension meetings, as well as a generally well-attended high school outreach program, AgFutures, which draws interested students from around the SJV. Tours of the KREC facility of all types typically have incorporated earlier air quality installations on site as key stops on the tram tours, or as featured subjects in public presentations. This purpose built facility was designed to enhance the outreach that was already occurring, and to increase and diversify the number of visitors.

During Fall 2001 the computer used to control the ozonation and to record and monitor physical parameters in each open-top chamber was upgraded to a more rapid processor and larger storage capacity. A second, large format monitor was added to the data handling computer, placed in the middle room of the mobile laboratory, so that large groups of visitors to the facility would be able to see simultaneous and real time ambient ozone in each of the chambers, and selected physiological parameters.

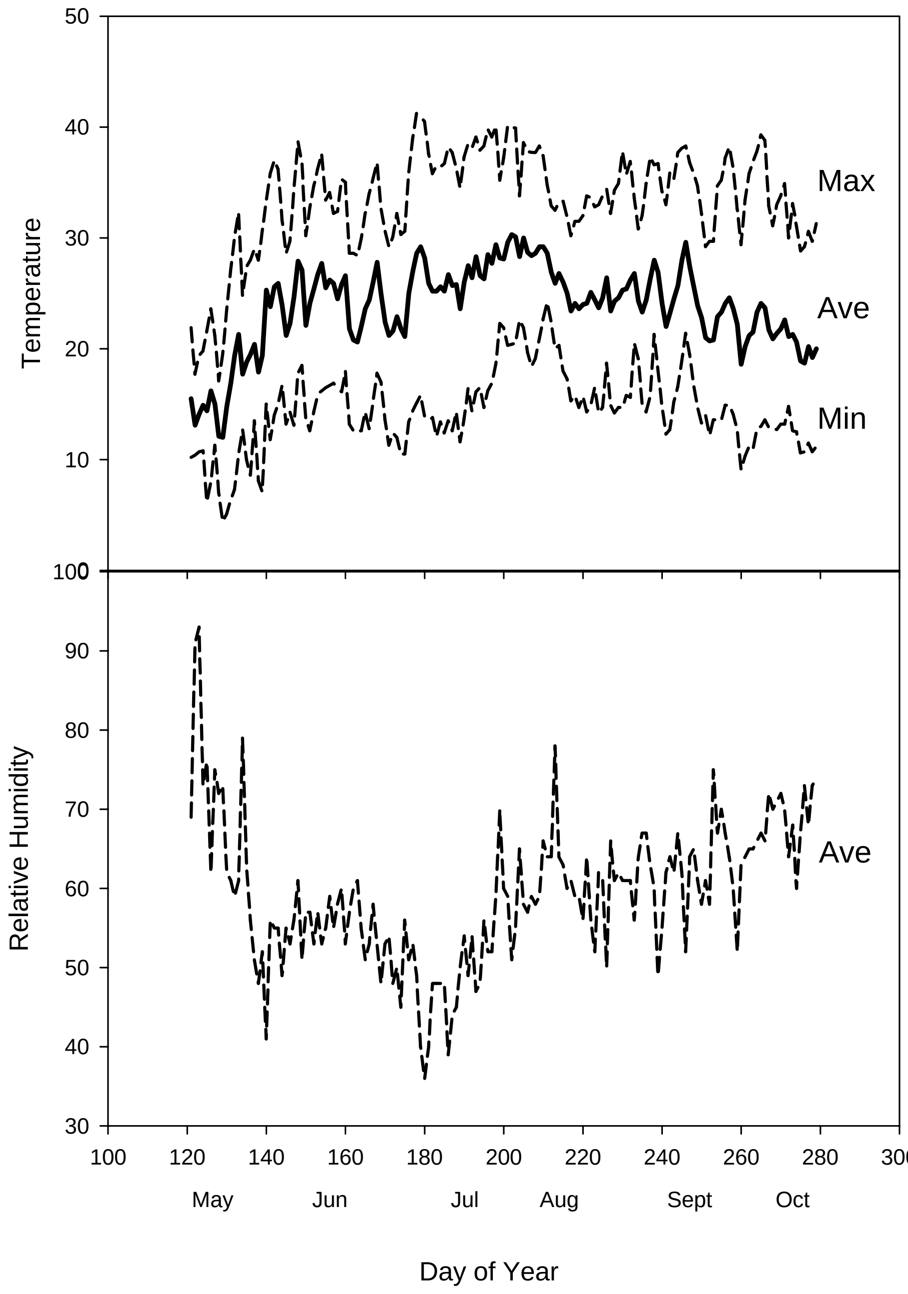
The web site was created initially using locally available skills at KAC. This included development of the cartoons for the children’s pages and the conceptual lay out. In later stages, and in preparation for taking the site live, the services of the University of California at Davis, Fruit and Nut Research and Information Center, were enlisted. This professional intervention late in site development, allowed some stylistic errors to be corrected, and imposed a consistency on the site that had been lacking.

RESULTS

Local Environmental Conditions

At the Kearney Research and Extension Center in Parlier, California, the daily temperatures during the growing season are above those expected to be optimal for kiwifruit production, but are well suited to cotton and melon, the perennial trees, and the summer annual ornamental species (Fig. 4, upper panel).

Fig. 4. Daily maximum, minimum and hourly average temperatures (^oC; upper panel) and average relative humidity (% RH; lower panel) at Parlier, California during growing season 2003.



Daily maxima frequently rose above 35C (Figure 4, top panel). High temperature conditions are linked meteorologically with episodes of high ambient ozone concentrations, at this location and throughout the arid western U.S.

Daily minimum temperatures were well above freezing during the May through October period. During the off-season (November through April) low radiation and cool daytime temperatures limited plant growth. Additionally, overnight freezing temperatures are not uncommon during the winter.

Relative Humidity is relatively low after the end of May. Average RH (Fig. 4, lower panel) reflects the occurrence of dew and associated saturated conditions in early morning hours throughout the season. Midday values of RH, however, are considerably lower, leading to large evaporative demand (see California Irrigation Management Information System [CIMIS] web-based information at http://www.cimis.water.ca.gov/). These are conditions that may exacerbate ozone-induced symptoms related to stomatal closure, oxidative foliar damage, and impaired root hydraulic properties. These interactions are described for cotton by Grantz (2003).

Ambient concentrations of tropospheric ozone are at damagingly high levels in the SJV (Figure 5). Elevated ozone preceeded the main portion of the growing season, with hourly concentrations over 80 ppb my mid-May.

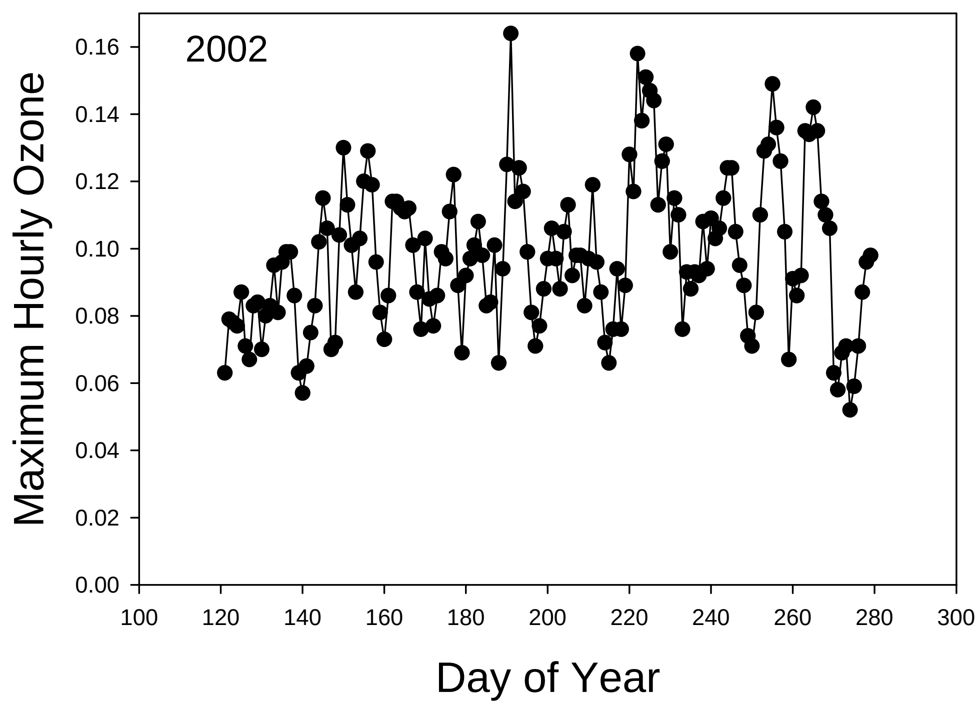


Fig. 5. Daily maximum hourly average ozone concentrations (ppm) at Parlier, California during the growing season of 2002.

Ozone Damage to Plants

An important component of the outreach potential of the OTC facility is the research equipment and instrumentation, and the presence of plants at all stages of development. These provide visual and physical/tactile demonstration materials. In addition, the scientific results that become available provide an important background for discussions of the broad range of air quality issues.

Cotton and melon. Pima cotton and muskmelon (cantaloupe) were the most successful demonstration species, even when grown in the near-hydroponic conditions of pots of sintered clay. In cotton, for example, both plant size, leaf area, and foliar symptoms were clearly associated with the level of ozone exposure (Figures 6 and 7).

Figure 6. Pima cotton was sensitive to ozone exposure. Here a plant subjected to charcoal filtered air showed no symptoms, and developed healthy, deep green leaves.

Figure 7. Pima cotton was sensitive to ozone exposure. Here a plant subjected to high ozone concentrations grew very poorly, exhibited a slow rate of development of new leaves, reduced leaf expansive growth, and symptom development, particularly on older leaves.

During the 2002 growing season the principal research objective was characterization of the impact of ozone on root morphology in cotton and melon. Results supported our earlier findings, obtained in other types of exposure environments, that ozone inhibited root hydraulic capacity. We also found new evidence that ozone inhibited phloem loading, which is the first step in moving photosynthetically produced sugars out of the source leaf, towards sink tissues such as roots following photosynthesis. By comparing cotton and melon, two species which load sugars into the phloem for long distance transport by different biochemical mechanisms, we further localized the impact on loading of diverse sugars.

During early 2002 the project released a research publication providing a new level of detail regarding the responses of root respiration to ozone. These findings were novel. A disconnect was demonstrated between photosynthesis which provides sugars to the roots, and root respiration which consumes the sugars.

In 2003 a colony of cotton aphids was developed to use in evaluating the role of ozone in changing sugar profiles in the plant transport fluids. Aphids provide a useful tool, because they feed exclusively on the transport fluids in the phloem, and thus provide a highly specific probe for the materials in the phloem sap. The colony of cotton aphids is now self-maintaining in a research greenhouse at KREC.

In mid-summer 2003 melon aphids (a biotype variant of the same species of cotton-melon aphids) were collected from a cantaloupe field site at KREC. A thriving colony of this biotype was established in the same greenhouse. The distinct colonies are contained in separate isolation cages.

Both populations have functioned efficiently in sampling phloem sap from their respective host species in the OTCs. Collection of honeydew (sap passing through the aphid gut with minimal modification) served as a surrogate for directly sampled phloem sap. These analyses confirmed previous studies showing that ozone exposure altered the ratio of sucrose to raffinose series sugars. This has important implications regarding the mechanism of ozone impact on plants. It also provided a unique scientific context in which to demonstrate the other ozone impacts in the OTCs, particularly on root growth (e.g. Figures 8-10).

These results, and our collaboration with analytical chemists utilizing the most sensitive chromatographic techniques, revealed the presence of many previously unknown constituents of phloem sap including some large polymers.

These results led to an invitation by the cotton industry to present the results at the 2004 Beltwide Cotton Conference. Industry is most interested in the implications for sticky cotton, resulting from aphid feeding and deposition of honeydew on developing cotton bolls and fiber. These possible interactions of ozone and the large commercial problem of sticky cotton are a previously unexplored impact of air pollution on a crop system.

Ozone impacts on the composition of the large sugars that we have identified are suggested but require further characterization.

Figure 8 (next page). Pima cotton was sensitive to ozone exposure. Here a plant subjected to charcoal filtered air showed no symptoms, and developed a healthy, extensive root system. Here the entire root system of an 8 week old plant was surgically divided into 12 subsamples for imaging and analysis of root morphology.

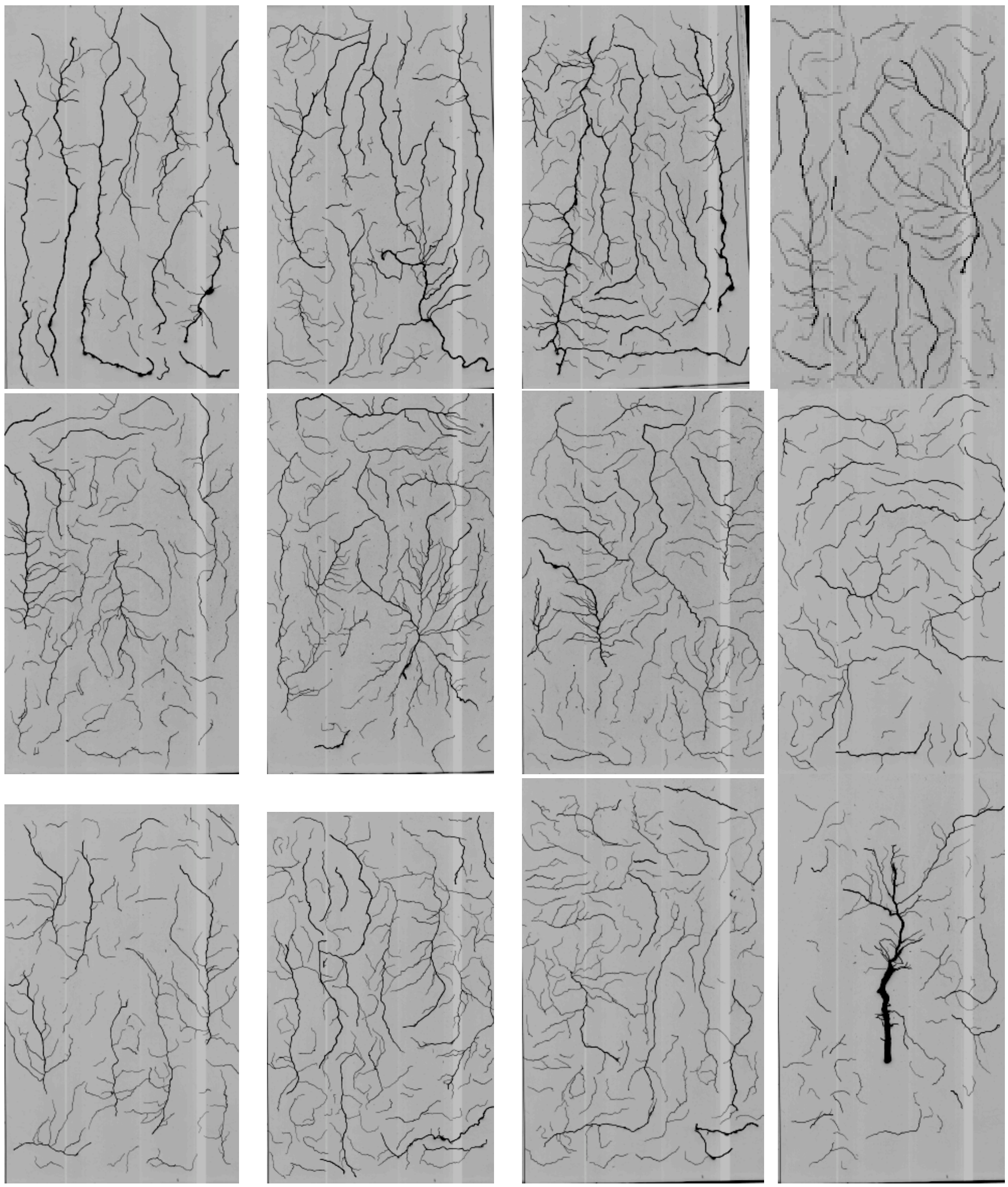


Figure 9. Pima cotton was sensitive to ozone exposure. Here a plant subjected to a moderate ozone concentration developed a healthy, but less extensive root system, than plants grown in charcoal filtered air. Here the entire root system of an 8week old plant was surgically divided into just 8 subsamples for imaging and analysis of root morphology.

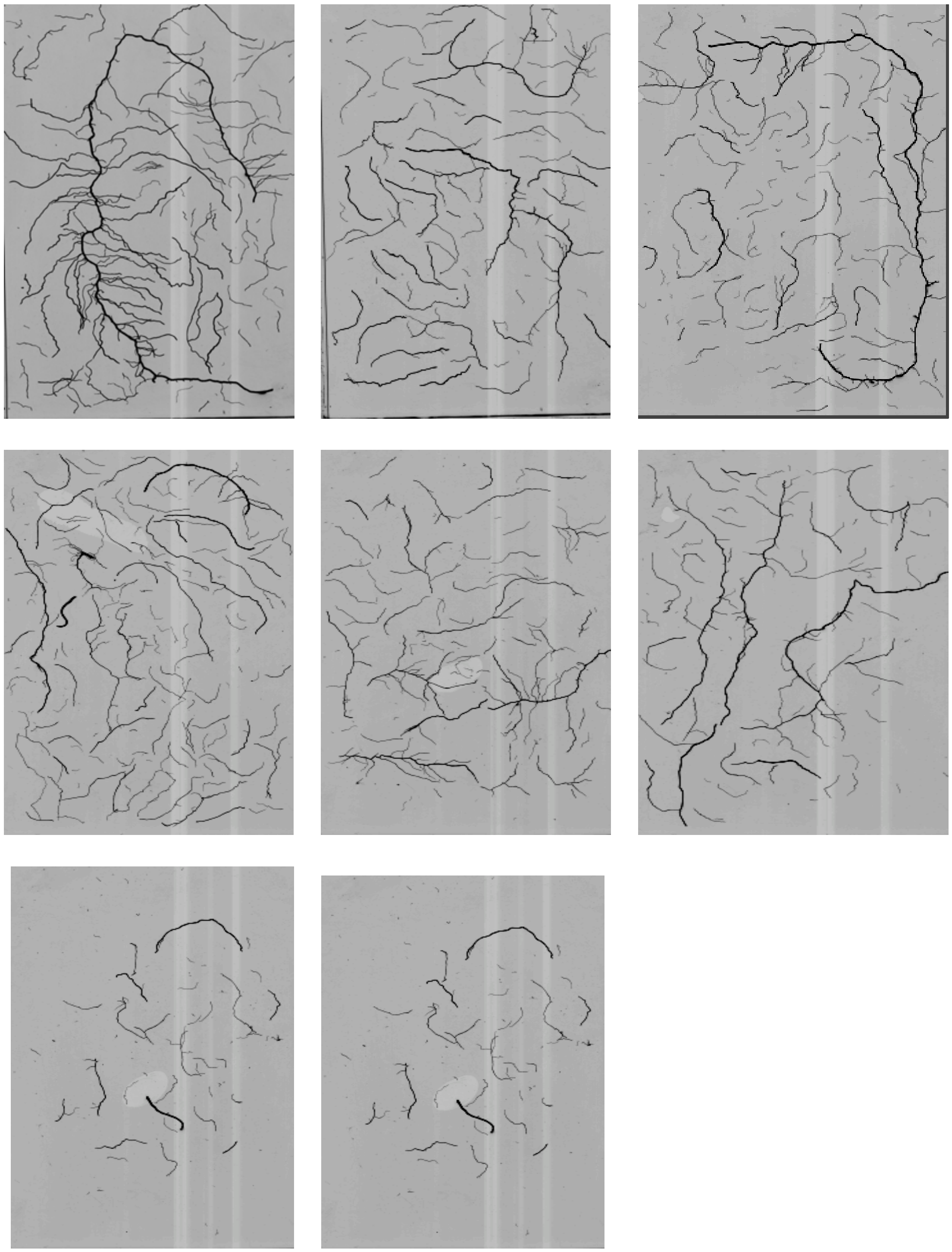
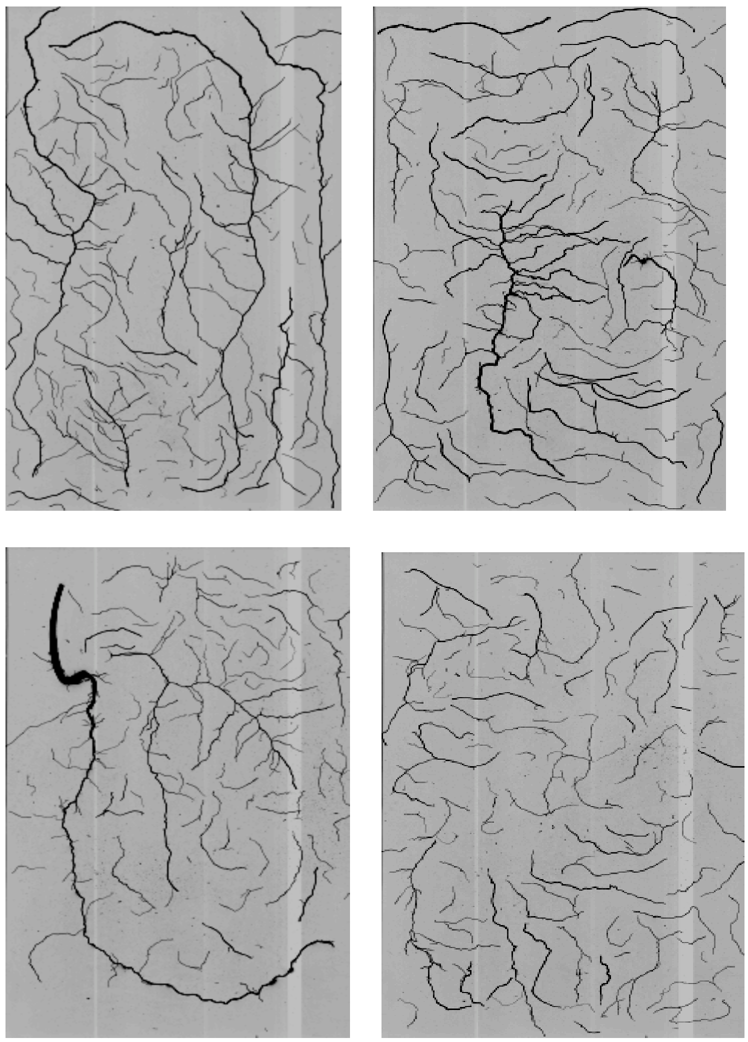


Figure 10. Pima cotton was sensitive to ozone exposure. Here a plant subjected to a high ozone concentration showed slight symptoms of ozone damage on the lower leaves, and developed a stunted and extremely weak root system, compared with plants grown in charcoal filtered air. Here the entire root system of an 8 week old plant was surgically divided into just 4 subsamples for imaging and analysis of root morphology.



Tomato. A number of new weed species, and some with increasingly competitive properties, are emerging in the San Joaquin Valley. The OTC demonstration site at KREC drew the attention of the regional Integrated Pest Management Farm Advisor for weed control. The effect of ozone on weed-crop interactions was investigated using the same cultural system as for the other plant species. A locally important cultuvar of tomato and locally collected nutsedge propagules were established in pots in the OTCs.

Figure 11. Kiwifruit did not exhibit pronounced symptoms of ozone exposure. These vines were exposed to high ozone concentrations, but showed no more symptoms than did the vines exposed to charcoal filtered air. They did exhibit reduced sucker development along the main stem.

Tomato plants grown in 2003 were relatively tolerant of ozone compared with Pima cotton or muskmelon. This property of tomato largely confirms previous studies of ozone-crop loss assessment. However, tomato growth was inhibited by ozone exposure to a modest extent. Nutsedge, on the other hand, appeared to be relatively sensitive to ozone. This was unexpected for a weedy plant. However, nutsedge is largely vegetatively (clonally) propagated. This reduces the rate of genotypic adaptation to emerging stresses such as ozone. Other weed species which are propagated by sexual means through seed dispersal are likely to exhibit greater ozone tolerance. Interest in these results by growers and Farm Advisors and Pest Control Advisors suggests that further research and demonstration in this area may be warranted from a research perspective, as well as of interest in generating interest in the outreach component of future projects of this type.

Kiwifruit. The presence of the Kearney OTC facility became known to a large commercial kiwifruit operation in Tulare County. They expressed concern regarding specific leaf symptoms which resemble ozone injury, and regarding a midday decline in carbohydrate production. These apparent symptoms were mostly observed with a new cultivar of kiwifruit introduced for extensive cultivation in the San Joaquin Valley.

An important feature of the ongoing OTC Demonstration Project was that ozone impacts on cotton and melon, which were readily available in the OTCs, could be quickly demonstrated to a group of consultants, growers and University of California Cooperative Extension personnel. This visual evidence, and background information suggested that further involvement could be useful.

At industry request kiwifruit plants were added to the research and demonstration species in the OTCs. The kiwifruit grew well, but exhibited leaf necrotic lesions at all levels of ozone. No specific visible symptoms of ozone damage could be identified, despite documented evidence of physiological impacts of ozone. For example, on these plants midday photosynthetic gas exchange (Fig.12, top panel) indicated a decline (not statistically significant over these limited measurements) between charcoal filtered and near ambient ozone. Surprisingly, assimilation increased with further doubling of the ozone exposure. These measurements were taken on the youngest fully expanded leaf. Therefore, it seems that this leaf position is occupied by younger leaves in the highest ozone treatment, relative to the other treatments. This was to be expected due to an accelerated rate of leaf senescence and population turnover (abscission). Future measurements on kiwifruit should be made over the entire life span of individual leaves, and over several leaf insertion levels to further characterize this inhibitory effect of ozone on kiwifruit productivity.

Stomatal conductance declined monotonically with increasing ozone on three measurement dates (Fig. 12, lower panel). It is noteworthy that the lowest conductance was observed in August, when temperatures were elevated and relative humidity low. Reduced stomatal conductance in the presence of ozone is a characteristic symptom of ozone damage.

The effect of ozone on stomatal conductance of both surfaces was striking. Although small in magnitude, conductance of the upper surface increased with increasing ozone, (Figure 13, upper panel) while the much larger conductance of the lower surface declined (Figure 13, lower panel). This resulted in the decline in whole leaf stomatal conductance observed above using the dual sided leaf cuvette (e.g. in Fig. 4, lower panel).

Figure 12. Photosynthetic and stomatal behavior observed with a steady state gas exchange system (LiCor 6400).

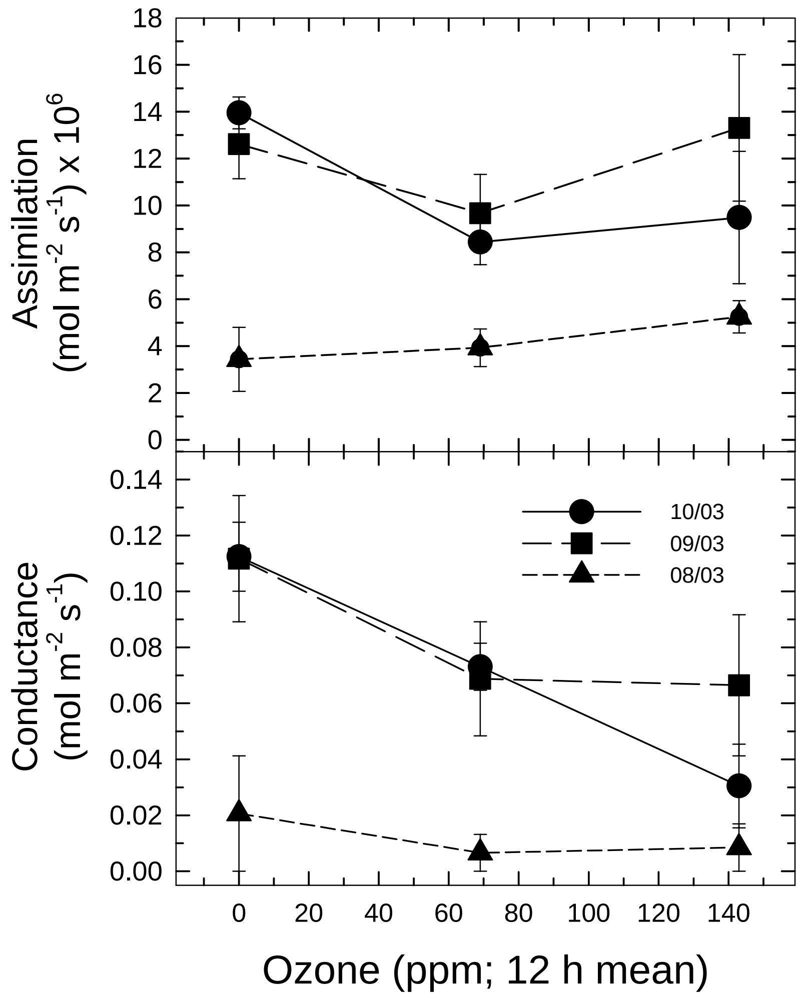
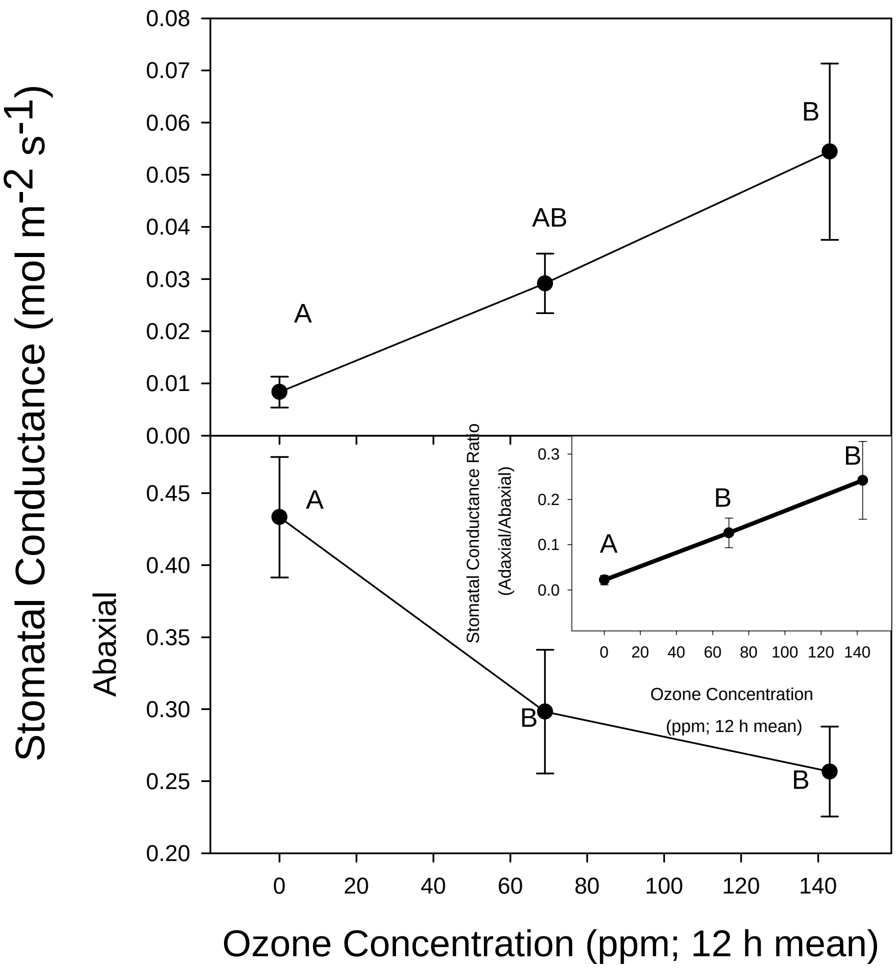


Fig. 13. Representative midday values of upper (top panel) and lower (bottom panel) stomatal conductance obtained with a single-sided, steady state porometer (LiCor 1600). Insert, ratio of upper to lower surface stomatal conductance. Data were analyzed as one way ANOVA, with n=12, 10 and 9, for low, medium and high ozone concentrations. Ratio data were transformed as [arcsin (square root)] prior to analysis.



Root health of the kiwifruit plants was not obviously impacted by the ozone treatments (Figures 14a-c). None of the treatments had extensive areas of necrotic roots, nor any sign of necrosis or decay. However, because these vines were obtained from a commercial nursery at about 1 year of age, the root systems had fully explored the pots prior to initiation of ozone exposure. Exposure beginning at plant establishment would be expected to identify more precisely any effects of ozone exposure on biomass below ground. Such impacts are expected based on results in these OTCs with cotton, melon and tomato.

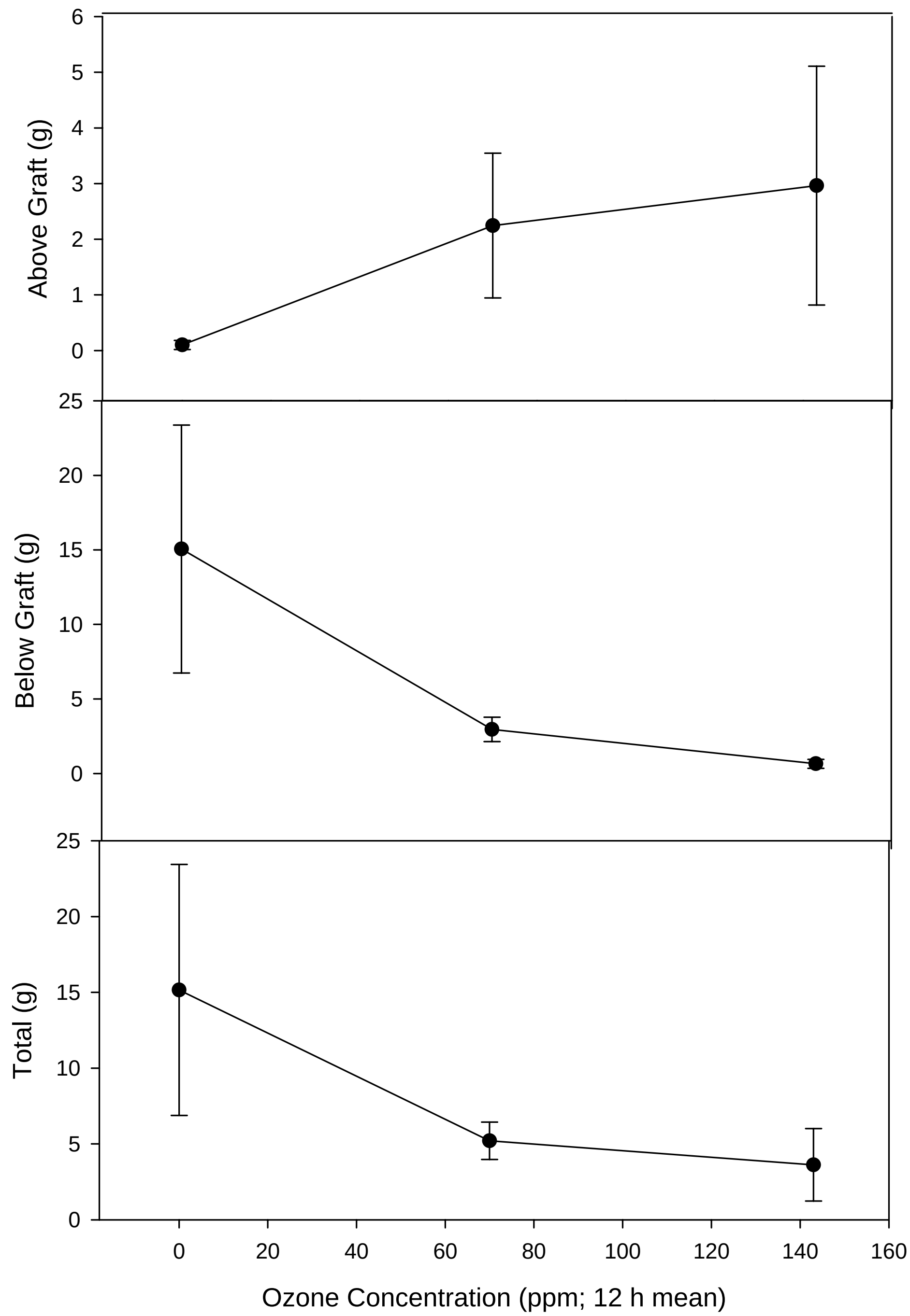
Fig. 14A. Photograph of root system obtained from kiwifruit plant exposed to Low Ozone (nominally 0.0 ppb, 12 h mean) at Kearney Agricultural Center.

Fig. 14B. Photograph of root system obtained from kiwifruit plant exposed to Medium Ozone (nominally 69 ppb, 12 h mean) at Kearney Agricultural Center.

Fig.14C. Photograph of root system obtained from kiwifruit plant exposed to High Ozone (nominally 143 ppb, 12 h mean) at Kearney Agricultural Center.

Production of weak vegetative shoots (suckers) from the lower main stem is a horticultural problem in kiwifruit. Ozone had a modest impact on sucker production. Early in the trial, all suckers were removed from the plants and discarded. At the end of the growing season, all suckers that had initiated during the ozone exposure period were again removed from the plants. Suckers emerging above the graft were separated from those originating below the graft and both were dried and weighed. Ozone reduced suckering below the graft (Figure 15, middle panel), while enhancing sucker production above the graft (Figure 15, top panel).

Fig. 15. Dry weights of prunings from suckers originating above the graft union (upper panel), below the graft union (middle panel), and total pruning weight (lower panel) Treatments were not significantly different.



Total biomass invested in sucker development was reduced by ozone exposure (Figure 15, bottom panel). Although treatment differences were visibly striking, and on average appeared to reflect substantial ozone impact, the results were sufficiently variable that results were not statistically significant between ozone treatments.

Other local crop species. Almond, grape, and peach, important local horticultural crops, did not perform well over extended periods in the OTCs, even when grown in high quality potting media. Off season (winter) cultivation of sweet peas was attempted, but was unsuccessful due to low available light in this foggy environment.

Outreach

In mid-year 2000 the Public Education group at the Air Resources Board in Sacramento was consulted with the goal of collaboration to produce outreach materials. A consensus was reached to begin development of a promotional video to attract visitors to the OTC facility, and to provide scientific and regulatory information to both visitors and school children in their classrooms. Despite considerable initial enthusiasm, it was subsequently determined that the ARB media staff would not be able to participate in developing the proposed video projects.

In future demonstration projects of this type it is suggested that such video products could make a potentially useful contribution. It was initially believed that this decision only represented a deferral of ARB participation. Ultimately the collaborative project was not revived.

The Community Education staff at the Fresno Bee, a prominent print media outlet in the SJV, provided outstanding cooperation. We received multiple copies of the Blue Sky-Brown Sky program, including numerous attractive handouts and souvenir items for students. These materials had been developed in collaboration between the Fresno Bee and the local air quality regulatory authority, the San Joaquin Valley Unified Air Pollution Control District. These materials were of great interest to the students who received them. Such materials remain an important component of outreach programs of this type.

Repeated attempts to enlist the public education staff of the San Joaquin Valley Unified Air Pollution Control District in collaborative outreach projects with this KAC Demonstration Project were unsuccessful. As our target clientele are similar, and geographic focus overlapping, this would suggest a potentially useful collaboration in future projects of this type.

A web site was developed to support the demonstration aspects of this project. This is hosted on the UC KAC server, and is linked through the websites of the U.C. Riverside Department of Botany and Plant Sciences and the U.C. Davis Fruit and Nut Research and Information Center.

The home page (Figure 16) provides a brief overview of the problem and three “clickable”, visually attractive, navigation buttons. Each button leads to material for a specific audience. The “Current Research” button leads to an entry page (Figure 17) with links to the publications associated with this project, and to photographic documentation of damage to plants caused by ozone and other pollutants. Links are provided to other air quality effects sites, notably those posted by the U.S. Department of Agriculture at North Carolina State University, Newcastle University in Britain, the California Air Resources Board, and U.S.E.P.A. Links.

“Valley Air Quality” provides regulatory information and a variety of archived and real time data assemblages of ambient pollutant concentrations locally and throughout California and the U.S. (Figure 18).

The children’s entry page (Figure 19) provides access to educational cartoons, appropriate links to other children’s websites, and resources for teachers.

Figure 16. Home page of the air quality effects web site. The site is hosted at the Kearney Agricultural Center, and may be accessed at:

http://airqualityeffects.uckac.edu

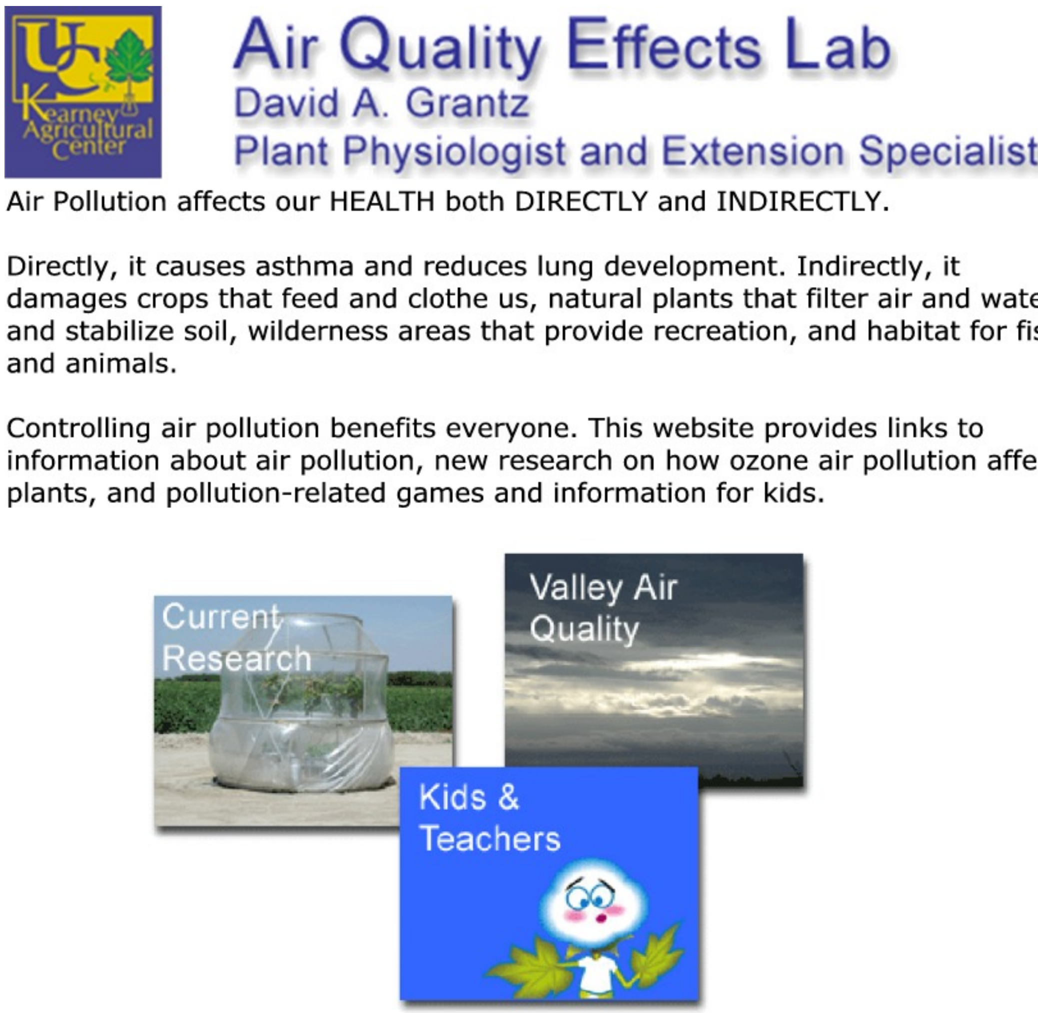


Figure 17. Current Research page of the air quality effects web site.

Air Quality Effects Lab
Current Research

Home | Current Research | Valley Air Quality | Kids & Teachers

Current Research Projects on Ozone Impacts on Plants

Links on this page:

- Research Summary
- Links

Links to related pages:

- Recent Publications
- Photographs

Research Summary

Ozone reduces plant growth, yield of horticultural and agronomic products, and beauty of ornamental vegetation. But the mechanism of ozone damage is not well understood. As a result, methods to protect plants are not available.

The **Air Quality Effects Laboratory**, in collaboration with other researchers in California, other states, and other countries, is working to understand the mechanism of ozone damage, to develop production methods to protect crops, and to identify targets for genetic improvement of plants.

It is well known that photosynthesis is damaged by ozone. It is less clear that this is the only or even the primary target of ozone action. Loading of newly photosynthesized sugars into the phloem for long distance translocation to roots and fruits is rapidly inhibited. Allocation of biomass among competing plant parts is disrupted by ozone, reducing root development and hydraulic conductance, and indirectly affecting leaf water relations and photosynthetic gas exchange. The **Air Quality Effects Laboratory** has shown that in Pima cotton (*Gossypium barbadense*) carbohydrate translocation is inhibited more than photosynthetic carbon assimilation following brief exposures to high concentrations of ozone.

Pima Cotton loads sugars into the phloem from the apoplast, which requires uptake of sucrose across a cell membrane. Muskmelon (Cucumis melo) loads an alternative type of sugar, mostly stachyose, from the symplast, which does not require uptake across a membrane. The **Air Quality Effects Laboratory** has found that the ratio of stachyose to sucrose in sink tissues, such as fine roots, increases with exposure to ozone in both types of plants. These data suggest that O3 inhibits apoplastic phloem loading of sucrose more than symplastic loading of stachyose. Future research must determine if specific transporter proteins are particularly sensitive to ozone and could be a target for genetic improvement.

Root development is inhibited by ozone. As a result the hydraulic capacity to provide the transpiring shoots with water is reduced. The **Air Quality Effects Laboratory** has used a model to show shown that this reduction in root capacity could reduce photosynthesis and plant water use. Total root biomass is reduced, the fraction of plant biomass in root tissues declines, and the number and branching patterns of roots is altered by ozone. Changes in internal anatomy of individual roots is currently under investigation.

Crops are sensitive to weed pressure. The **Air Quality Effects Laboratory** has demonstrated that purple nightshade, a strong competitor to Pima cotton, is an even stronger competitor in the presence of ozone exposure. While total productivity of nightshade plus cotton declined with increasing ozone concentration, the ratio of cotton to nightshade biomass declined dramatically. Yellow nusedge is another important weedy competitor. In trials with tomato (Lycopersicon esculentum) both tomato and nutsedge were sensitive to ozone and the relative competitiveness of nutsedge did not increase. In ongoing trials with cotton, which is more sensitive to ozone than tomato, competitiveness may have increased.

Links

- UC Berkeley Digital Library Project - 25,000 photographs of plants with search tools based on color, common or scientific name, habitat and more
- Air quality standards (pdf) - current state and federal
- CARB - ozone and its damaging characteristics
- USDA-ARS: Ozone damage to crops - information and photographs
- USEPA - static and animated ozone maps of the U.S. and Air Quality Index
- UNECE-ICP: Ozone damage to forests - in Europe
- University of Newcastle air pollution - ozone effects on plants
- Ozone damage to tobacco - symptoms in cultivar Bel W3.
- A model indicator system
- Ozone damage to common plants - in Greece

Documents with the designation (pdf) are in Adobe Portable Document Format (PDF) and may be downloaded, viewed, and then printed by using Adobe Acrobat Reader©, which is freely available from the Adobe site. Download Adobe Acrobat Reader.

Figure 18. Current Research page of the air quality effects web site.

Air Quality Effects Lab
Valley Air Quality

Home | Current Research | Valley Air Quality | Kids & Teachers

The San Joaquin Valley of California is a large geographic basin with a serious air pollution problem. It is subject to high summer temperatures and solar radiation. It has restricted air exchange because of surrounding mountains and low wind. Its population is growing and industry, vehicles and miles traveled are all increasing. The Valley could pass Los Angeles in the race to the bottom of air quality. It is already one of the three most polluted air basins in the United States.

Ozone generation and accumulation are of particular concern to agriculture, natural plants, and human health. Emissions of hydrocarbons and oxides of nitrogen from many sources lead to ozone air pollution. The Valley has been a very productive agricultural region, but this could be threatened by these increasing ozone concentrations. On many days, models suggest that yield could be reduced by 10-20% in many field and tree crops.

Air Pollution Levels

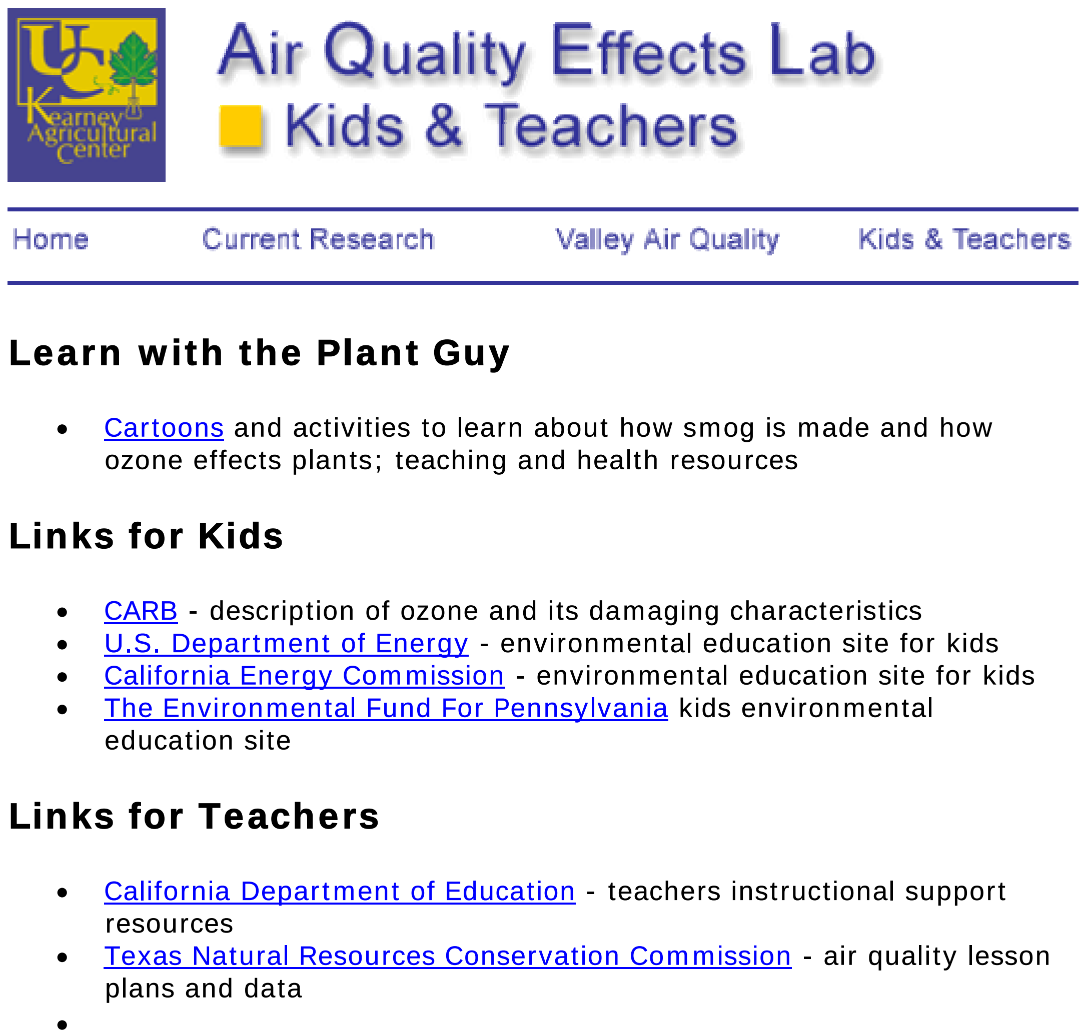
- California Air Resources Board - current and archived California air quality data for specific locations
- USEPA - static and animated ozone maps of the U.S. and Air Quality Index
- USEPA Air Now - daily simulation maps of ozone concentration. Select "San Joaquin Valley" for local conditions as they develop over time
- San Joaquin Valley Maps - most current air quality index information

Background Information

- Ozone Damage to Plants - Air Quality Effects Lab
- Air quality standards (pdf) - state and federal
- CARB - description of ozone and its damaging characteristics
- Description of particulate matter and its damaging characteristics - the CARB regulatory process
- USEPA Ozone impacts on health - and how to protect yourself
- Federal Clean Air Act of 1990 - explanation
- California Air Resources Board - many air quality information sites
- USEPA - global warming

Documents with the designation (pdf) are in Adobe Portable Document Format (PDF) and may be downloaded, viewed, and then printed by using Adobe Acrobat Reader©, which is freely available from the Adobe site. Download Adobe Acrobat Reader.

Figure 19. Current Research page of the air quality effects web site.



Air Quality Effects Lab
Kids & Teachers

Home | Current Research | Valley Air Quality | Kids & Teachers

Learn with the Plant Guy

- Cartoons and activities to learn about how smog is made and how ozone effects plants; teaching and health resources

Links for Kids

- CARB - description of ozone and its damaging characteristics
- U.S. Department of Energy - environmental education site for kids
- California Energy Commission - environmental education site for kids
- The Environmental Fund For Pennsylvania kids environmental education site

Links for Teachers

- California Department of Education - teachers instructional support resources
- Texas Natural Resources Conservation Commission - air quality lesson plans and data
-

A large section of the web site is devoted to children. Animated presentations in Shockwave and Flash formats demonstrate the production of ozone in the atmosphere, and ozone damage occurring to leaves of cotton. Manipulated digital imagery is used of actual damaged and undamaged leaves of Pima cotton. These cartoons may be of interest to others developing similar web presentations.

The children's section of the web site also provides information on the components of air pollution, the health effects of specific pollutants, as well as a list of 50 things that students and other citizens can do to help decrease the amount of air pollution. We have also posted curriculum developed by ARB, so that teachers can download information to use in their classrooms, either as stand alone modules or preparatory to visiting the OTC facility at Kearney. In order to appeal to children an animated figure serves as a guide throughout this section of the site see "Kids and Teachers" button(Figure 16).

Tours and Direct Demonstration. A substantial amount of outreach was conducted by virtue of the maintenance of this OTC facility. However, the amount of direct outreach that was achieved was considerably less than expectations during the exceptionally difficult economic conditions that characterized the period of project activity. Visitor numbers were markedly reduced at KREC, and the AgFutures program was cancelled for the first time ever. The contentious state of air quality regulatory activities with respect to agricultural interests also reduced interest among the target population.

The "AgFutures" Day had been an annual occurrence at Kearney for many years. It typically brought several hundred high school students from around the Valley for tours of the Kearney facility, speeches, and conversations with practicing scientists. For the past several years AgFutures has included a visit to the KAC Air Quality Laboratory Facility. During 2001 we toured 307 high school students from 33 high schools from around the San Joaquin Valley, through the OTC facility. Interest level was high, and many interesting questions were asked and intense discussions occurred. High school student internships with the Air Quality Laboratory developed from these tours. Unfortunately, in subsequent years, tours of the Kearney facility of all types were generally reduced, and the AgFutures event was cancelled in 2002 and 2003 due to low registration by high schools.

The OTC demonstration facility became known to local agriculture teachers. A half-day tour of the OTC site was conducted for 10 members of the Reedley College faculty of agriculture. This group of agricultural specialists provided considerable opportunity for demonstration, and an extensive question and answer period. The promise of ongoing collaborations, and visits by future Community College students to the OTC facility emerged from the interaction. These connections provide a powerful conduit to the target community for information on air quality initiatives.

A public relations specialist was sent from The University of California, Division of Agriculture and Natural Resources, to tour and to report on the OTC facility at KREC. An electronic article was released, including photographs of the site. This material was featured on the Division web site, disseminating information on the ozone effects problem and our research efforts to understand the mechanism.

During this intense period of press coverage of the air quality issues of the San Joaquin Valley, several telephone and site interviews were conducted by print and television reporters. The Valley Voice newspaper toured the OTC site and published an article about the ozone problem and the OTC outreach effort in August 2002.

A reporter and a photographer from the Fresno Bee toured the OTC site, and took photographs. The discussions provided background for a series of articles on the air quality problem in the San Joaquin Valley that ran in the Fresno Bee over an extended period. A feature article on the site and our research and extension efforts was discussed but did not ultimately yield a published article. These articles in the Fresno Bee have been influential, and the issues of air quality in the Valley are now much higher profile than before, with occasional reference to the research and demonstration work at Kearney Agricultural Center

In Fall 2002 a reporter from the Vacaville Reporter conducted an interview regarding the OTC facility. Discussions centered on the reality and magnitude of crop loss in the San Joaquin Valley.

A planner for CalTrans called for the most recent data on crop loss due to ozone.

A reporter for the Associated Press toured the site.

During June 2003 the OTC demonstration facility was used to educate a large international horticultural group. Air quality issues were a novel consideration for many growers. As local and international growers are now planting extensive kiwifruit vines in Tulare County, there was considerable concern expressed that ozone may be causing certain leaf symptoms. A second, more focused tour involving local and international consultants, and a University of California Cooperative Extension Specialist, was made of the OTC facility to get an appreciation of authentic ozone symptomology. Further demonstration work with kiwi plants resulted from this tour, as noted above.

Concern among the large commercial kiwifruit operations in Tulare County about specific leaf symptoms which resemble ozone injury, and about a midday decline in carbohydrate production observed with a new cultivar of kiwifruit, led to their pursuit of closer coordination with the OTC facility. Initial demonstrations of ozone impacts on cotton and melon, were later expanded to include symptom evaluation on kiwifruits provided by the industry. Advisors from the San Joaquin Valley and from the parent organization in New Zealand have visited the Kearney OTC facility on several occasions to study the ozone impacts. The unusual symptoms of altered vegetative sucker development have been of particular interest. This observation, as well as a clear decrease in the life-span of photosynthetically-active leaves, has aroused considerable interest among the growers. At industry request, we are now planning further demonstration work in the OTC with this new cultivar of kiwifruit. This is exactly the type of mutually beneficial interactions that make a combination of research and demonstration such a potentially powerful outreach tool.

DISCUSSION

The research on mechanisms of ozone injury to key crops provided demonstration materials, as well as material for public and scientific presentations. The availability of such information drew a modest amount of interest from the media and from local academic institutions. Several media tours were conducted, and a few featured articles appeared, along with many other instances of background information. This increased the effectiveness of local reporting on air quality issues during a period of intense media scrutiny of air quality regulatory activity in the SJV. This intensity in many ways contributed to the lack of agricultural participation in the program, as attitudes hardened and the population polarized. Research with cotton and melon plants, both grown extensively in the SJV, yielded quantitative characterization of root system inhibition by exposure of the plant shoot to ozone. Results indicated that root respiration and oxygen consumption increased with ozone exposure. This suggests that a damaging signal is transferred from shoot to root, because the roots themselves are not exposed to ozone and photosynthesis declined while root respiration increased. This result may contribute to discovery of the mechanism of ozone damage to plants. Results also showed that the restriction of root development is largely confined to the very finest roots, which is particularly damaging since these roots absorb water and nutrients. Kiwifruit leaves did not exhibit symptoms of ozone damage. No visual symptoms, i.e. of discoloration, stippling, nor bronzing, were observed at any concentration of ozone. Overall health of the plants was only moderate, with considerable marginal leaf necrosis in all ozone treatments. Photosynthesis was still inhibited. Additionally, kiwifruit stems, which produce abundant undesirable shoots did not do so after shoot exposure to ozone. Basal sucker development declined from abundant to nearly absent with increasing ozone concentration. There was an indication of greater suckering from above the graft union in the higher ozone treatment than in lower ozone concentrations. A more rapid leaf turnover and consequent reduced leaf life span was clearly observed in the high ozone treatment, and was suggested in the moderate ozone treatment. Stomatal conductance declined with increasing ozone concentration, as expected. However, photosynthetic carbon assimilation declined from the charcoal filtered treatment to the moderate ozone treatment, but then increased with further increase in ozone concentration. This latter observation is anomalous, and is likely due to inadvertent assay of younger leaves in the high ozone treatment than in other treatments, due to the greater turnover of leaves noted above. Roots in all treatments appeared healthy, though modest ozone impacts on root development could not be ruled out.

Useful outreach materials and a continuing physical and web-based presence were established. Substantial research results were obtained. As the current CARB-supported program concludes, another program for local school children, Orchard Odyssey, is being revived and further developed. This program will carry forward many of the initiatives of this program, utilize many of the resources created by this program, and will continue to feature air quality as one focus of student activities.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The air quality demonstration components will be housed under glass rather than in field OTCs in future years, to allow for year round demonstration. This addresses a significant limitation of the current program. Most agricultural meetings occur during the winter, when farm operations are less demanding.

Future programs of this type should continue to incorporate a research agenda as well as a demonstration and outreach component. Both missions should be adequately supported to insure success. The continuing existence of such programs in both urban and rural environments is desirable, making a potentially useful contribution to emerging political consensus on air quality regulation.

Kiwifruit exhibits several responses to ozone exposure, some of potential horticultural importance. It lacks the clear visual symptomology that would allow ready diagnosis of ozone injury in the field. Yet several physiological responses are apparent. Ozone-reduced gas exchange may inhibit potential productivity. Further work is required to evaluate the magnitude of such reduction under ambient conditions. The associated stomatal closure may have a slight benefit of reducing transpirational water loss.

Elevated ozone appears to suppress basal suckering of the potted plants. As sucker removal is labor-intensive, if this effect is significant under field conditions it could be of economic significance. Of greater interest, the altered sucker development appears to indicate other physiological alterations due to ozone exposure. These may involve carbohydrate fluxes (see Grantz and Farrar, 2000, for ozone effects on phloem loading), root vitality and hydraulic properties (see Grantz and Yang, 1996 for ozone effects on root properties), carbon supply associated with photosynthesis, or other unknown impacts.

At the conclusion of this preliminary observational study, it is not proven that ozone poses a large threat to kiwifruit production in the San Joaquin Valley of California. Enough evidence was obtained, however, to suggest that this is a real possibility, and further investigation may be warranted. The involvement of the kiwifruit industry is emphasized because this type of grower interest and continued involvement in air quality research and demonstration efforts is a good example of the intended consequences of this project. Further projects of this type will lead to greater awareness of air quality issues among an increasing segment of the target (i.e., regulated communities).

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PUBLICATIONS ORIGINATING FROM THE PROJECT

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D. A. Grantz and A. K. Murray. (2004) Impact of ozone air pollution on phloem transport of carbohydrates in cotton. Physiology Research Conference of the 2004 Beltwide Cotton Conferences, San Antonio, TX January 5-9, 2004.

APPENDIX I.

TEXT OF PUBLICATIONS BASED ON DATA FROM THE PROJECT

Publication 1. Appearing in the Proceedings of the National Cotton Council, Beltwide Cotton Conferences. San Antonio, TX, 2004.

OZONE AFFECTS COMPETITION BETWEEN COTTON AND NUTSEDGE

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Abstract

Cotton production in the San Joaquin Valley and elsewhere is threatened by new and increasingly recalcitrant weeds, and by increasing ozone air pollution. Interactions are not well understood. In field exposure chambers with potted plants we found that plant growth was reduced in both cotton and yellow nutsedge when plants grown alone were exposed to high concentrations of ozone. When the two species are grown together the effect is compounded, particularly near ambient ozone concentrations. As tropospheric ozone levels increase, yield of cotton will be reduced due to oxidant damage and competitiveness of cotton with respect to weedy species will decline. Greater use of herbicides may result unless more ozone resistant cotton cultivars are developed.

Introduction

Ozone air pollution (O_3) reduces yields of adapted upland ***(****Gossypium hirsutum* L.) cotton cultivars by about 20% in the San Joaquin Valley (SJV) of California (Grantz & McCool 1992; Olszyk *et al.* 1993; Oshima *et al.* 1979; Temple *et al.* 1988a). Pima (G. *barbadense* L.) cotton cultivars are impacted even more (Grantz & McCool 1992; Olszyk *et al.* 1993). Ozone affects cotton through direct oxidant damage to physiological processes and ultimately to yield (Gimeno et al., 1995; Grantz, 2003).

Cotton production in the SJV is also challenged by competition from weeds. Weed control is a major cost in cotton production, with herbicide application to 60% of California cotton acreage (DPR, 2002). Herbicide application is becoming increasingly restricted (Szmedra, 1997), but prevents an approximate 60% loss of productivity in SJV cotton (NCFAP, 2000). A total of 413,418 lbs of Glyphosate (Round-up) was applied to 412,540 acres of the 690,000 acres of cotton planted in CA for weed control in 2002 (DPR, 2000).

Yellow nutsedge (*Cyperus esculentus* L.) is one of the world's most problematic weeds (Holm et al., 1991), particularly well-adapted to irrigated field and row crops (Holm et al., 1991; Mulligan and Junkins, 1976) including cotton under SJV conditions.

Much of the cotton acreage in the SJV is subject to increasing concentrations of O_3. Little is known of crop-weed interactions under elevated O_3 (Fuhrer and Booker, 2003; Ziska, 2002). The ability of nutsedge to compete with cotton under elevated O_3 has not been explored.

Materials and Methods

Plant Material

Cotton (cv. Pima S6) was grown from seed in 9-l tapered plastic pots (Treepot; Hummert International, Earth City, MO), filled with 6-40 mesh sintered clay (Quicksor, A & M Products, Taft, CA).

Yellow nutsedge seedlings, approximately 6 cm tall with 2-3 leaf blades were collected from field sites near Parlier, California. Seedlings were washed to remove soil, trimmed if necessary to a single tuber, and transplanted to pots containing cotton on July 18, 2003. Several seedlings were planted to each pot, and thinned five days later to desired densities of 1, 2, or 3 plants pot^{-1}. Additional pots contained only a cotton plant or only a nutsedge seedling.

Pots were randomly assigned to an Open Top Chamber (OTC) for exposure to one of three different concentrations of ozone. The pots were automatically irrigated to run-through up to three times a day as required by the weather. A complete fertilizer (Miracle Gro; Scotts Miracle-Gro Products Inc., Port Washington, NY) was applied at 1.3 g l^{-1} weekly.

Ozone Exposure

Experiments were conducted in the OTC (3.1 m diameter x 2.4 m height; Heagle et al., 1973) exposure facility at the University of California, Kearney Agricultural Center, Parlier, CA (103 msl, 36.598 N 119.503 W). Ozone was generated by corona discharge (Model G22; Pacific Ozone Technology, Brentwood, CA) from oxygen (Model AS-12; AirSep Corporation, Buffalo, NY). The daily timecourse of O_3 concentration was regulated in a single OTC using a dedicated O_3 monitor (Model 49C, Thermo Environmental Instruments, Franklin, MA) interfaced to a computer for feedback control, as described previously (Grantz et al., 2003). The low O_3 (LO3) regime was charcoal filtered (CF) and was nominally O_3-free (actua 12 hm = 15.9 ppb). The medium O_3 (MO3) regime approximated the diurnal profile and maximal concentration observed on exceptionally polluted days at this location (actual 12 hm = 80.6 ppb). The high O_3 (HO3) regime was approximately 1.9-fold greater than the MO3 at each time point (actual 12 hm = 153.6 ppb).

Experimental design

The experiment was arranged as a split plot with four replications. Ozone regime (LO3, MO3, HO3) was the main plot and nutsedge density (0, 1, 2, 3 plant pot^{-1}) was the subplot. Each individual pot was an experimental unit, with four replicates of each subplot. Data were analyzed using PROC GLM (SAS Institute, 1990). Additional single degree of freedom contrasts were conducted of biomass parameters in both species grown with and without competition.

Growth measurements

Weekly measurements were taken of plant height in cotton. Both species were destructively harvested at 2 months after planting. Cotton plants were separated into leaves (laminae plus petioles) and stems. The number of leaves attached to the cotton plants at harvest was counted. Total leaf area was measured by a LI-3000 area meter (LI-COR). Leaf area of the nutsedge plants was not measured, Shoot biomass was stored at 4ºC until (1-2 days) then dried to constant weight in a forced-air oven at 70ºC.

Nutsedge tubers were separated from the root mass, counted, and dry weight determined. The cotton and nutsedge roots were intertwined in pots containing both species, so dried biomass of the combined root system was determined. As considerable sintered clay growth medium adhered to this root mass, each sample was combusted (Thermolyne Corp., Dubuque, IA) at 800ºC, leaving only the sintered clay medium and a negligible amount of ash. Total root biomass was then determined as the difference between the root dry weight and the ashed weight of the sintered clay.

The root:shoot biomass ratios for both species grown alone (without competition) were calculated.

Leaf area ratio (LAR) and specific leaf weight (SLW) of the cotton plants were calculated as total leaf area/total shoot dry weight and total leaf area/total leaf dry weight, respectively. Similarly, leaf weight ratio (LWR) was calculated as total leaf dry weight/total shoot dry weight.

Results and Discussion

Growth of cotton plants was negatively impacted by exposure to ozone. Plants were significantly shorter (Table 1) and produced significantly fewer leaves and less biomass (Table 1). This was observed in shoot (Fig. 1a; open circles) and root (Fig. 1b; open circles), particularly at the highest ozone exposure (HO3). Shoot biomass was reduced by about 80% at HO3. In these experiments, shoot biomass was not reduced at MO3, though root biomass declined by a modest amount at this exposure.

Growth of nutsedge, in the absence of competition from cotton was similarly affected (Table 1; Fig. 2a), with little impact (slight increase) on shoot biomass at MO3 but substantial inhibition at HO3, relative to LO3. Root biomass declined slightly from LO3 to MO3, and substantially at HO3 (Fig. 2b).

In cotton plants, with and without nutsedge, the biomass was reduced to the same extent (Figure 1a). While the interaction of O_3 x weed density was not significant (not shown) it is clear (Fig. 1a) that shoot biomass at near-ambient O_3 concentrations (MO3) was reduced to a much greater extent than in clean air (LO3). In contrast, at very high O_3 concentration (HO3), O_3 reduced cotton growth to such an extent that the presence of nutsedge had no further effect. The root biomass decreased in all pots containing cotton and nutsedge as the concentrations of ozone increased (Figure 1b).

Cotton grown with a single nutsedge plant (Fig. 1b; 1:1, pale grey symbols) exhibited combined root biomass that was equal at all ozone concentrations to the sum of the root biomass of the two species grown separately. However, under these conditions, shoot biomass of cotton (Fig. 2a; 1:1, pale grey symbols) was inhibited by a single plant of nutsedge.

Additional nutsedge plants contributed less than additively to combined root biomass (Fig. 1b) but had no further effect on shoot biomass of cotton at MO3 or HO3 (Fig. 1a).

The addition of 1 or 2 nutsedge plants to the pots of cotton grown alone had no effect on cotton plant biomass at LO3. Addition of a third nutsedge plant depressed shoot biomass of cotton by a small amount at LO3. However, cotton plants were not able to tolerate the addition of 1 nutsedge plant when exposed to MO3 or HO3 growing conditions. Cotton growth was hindered by ozone, and near ambient conditions was further impacted by competition with nutsedge. This indicates that cotton must be kept weed free to be grown in areas where ozone levels are increasing.

Plant growth is reduced in both cotton and nutsedge when either is exposed to high concentrations of ozone. When the two plants are grown together the effect is compounded, particularly near ambient air pollution conditions. As tropospheric ozone levels increase, yield of cotton will be reduced and competitiveness of cotton with respect to weedy species will decline. Greater use of herbicides may result unless more ozone resistant cotton cultivars are developed.

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Table 1. Analysis of variance of the effects of ozone exposure and competition on growth parameters in cotton and nutsedge.

ANOVA Source of variation	**P-value**	
	Cotton	Nutsedge
Plant height		
Ozone	0.0002	--
Weed presence	0.1033	--
Shoot biomass		
Ozone	0.0001	0.0181
Weed presence	0.0049	0.2042
Number of leaves		
Ozone	0.0001	--
Weed presence	0.0005	--
Leaf area		
Ozone	0.0002	--
Weed presence	0.0195	--
Root weight		
Ozone	0.0010	0.0381
Tuber number		
Ozone	--	0.2807
Weed presence	--	0.4451
Tuber weight		
Ozone	--	0.2194
Root:Shoot ratio		
Ozone	0.1101	--

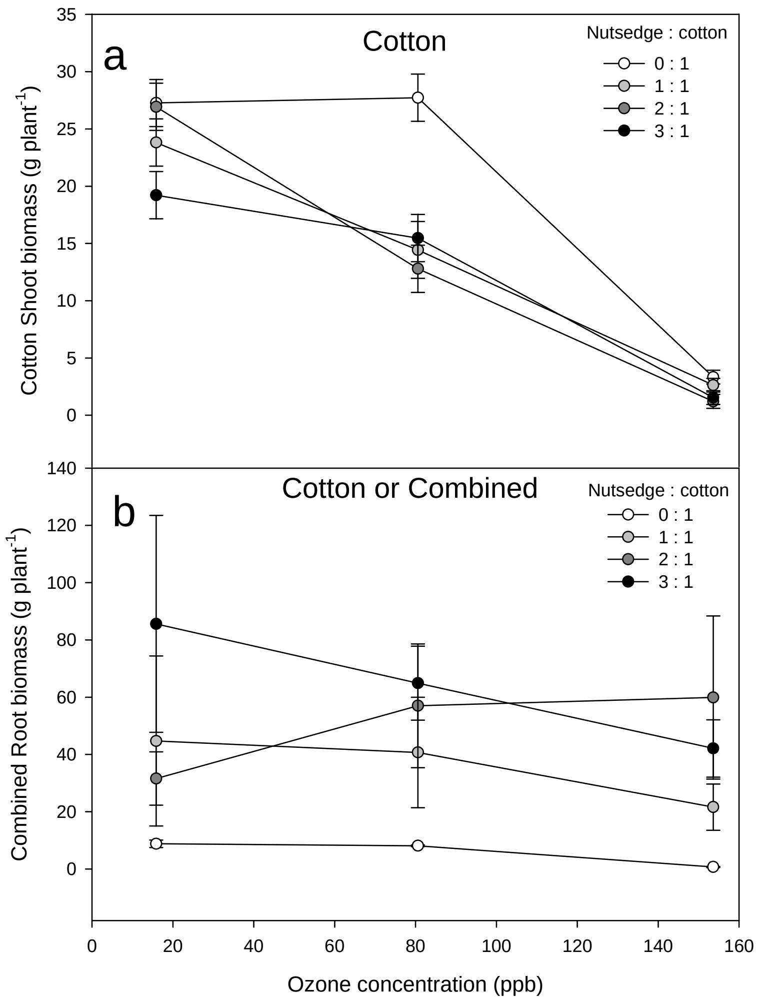

Figure 1. Effect of ozone exposure on (a) cotton shoot and (b) cotton or combined cotton plus nutsedge root biomass shown as means ± SE

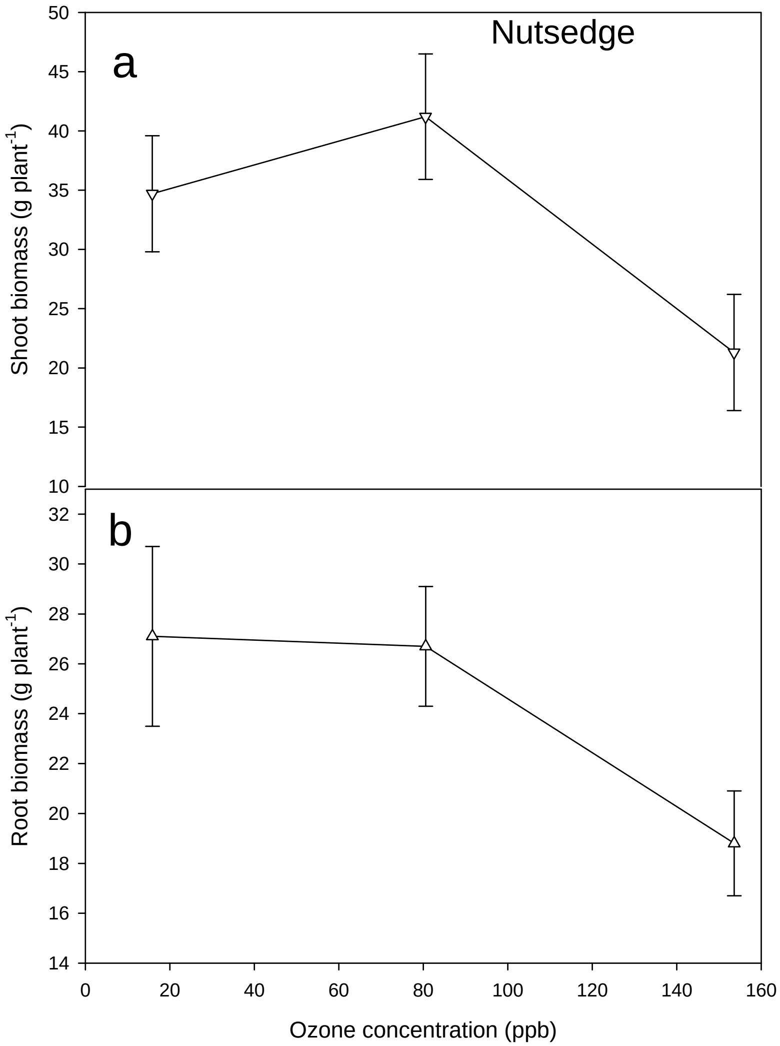

Figure 2. Effect of ozone exposure on (a) shoot and (b) root biomass of nut sedge grown alone, shown as means ± SE.

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Effect of Ozone on Phloem Transport in Cotton

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ABSTRACT

Pima cotton was grown in field exposure chambers exposed to a range of ozone concentrations. The ratio of sucrose to raffinose series sugars was examined in source and sink tissues and in aphid honeydew as a surrogate for phloem sap. The profile of sugars did not change substantially in source leaves. The ratio of sucrose to raffinose series sugars (raffinose plus stachyose plus verbascose) declined significantly in fine root tissue with increasing ozone. The composition of honeydew also declined, though not as dramatically as fine root tissue. These results are not conclusive but are consistent with the hypothesis that ozone alters the apoplastic loading of sucrose into the phloem more than it alters the symplastic loading of raffinose series sugars.

Abbreviations: 12 hm, 12 hour daylight (08:00 – 20:00) mean ozone concentration; CF, Charcoal filtered air; g_s, stomatal conductance; gfwt, grams fresh weight; HO3, high O_3 concentration; LO3, low O_3 concentration; LAR, leaf area ratio (total leaf area/total plant dry weight); LSD, Fisher's Protected Least Significant Difference; LWR, leaf weight ratio (total leaf dry weight/total shoot dry weight); MO3, medium O_3 concentration; OTC, open-top chamber; SLW, specific leaf weight (leaf area/total leaf dry weight).

Introduction

Tropospheric ozone (O_3) is a regional problem, and poses the greatest threat to vegetation of any air pollutant (Krupa *et al.*, 2001; Krupa and Kickert, 1989; Krupa and Manning, 1988). Damage to crops in the United States has been estimated at several billion dollars per year (Heck et al., 1983; Adams et al., 1988). O_3 reduces yields of adapted upland ***(****Gossypium hirsutum* L.) cotton cultivars by about 20% in the San Joaquin Valley (SJV) of California (Grantz & McCool 1993; Olszyk *et al.* 1993; Oshima *et al.* 1979; Temple *et al.* 1988). Pima (G. *barbadense* L.) cotton cultivars such as S-6 are impacted even more (Grantz & McCool 1993; Olszyk *et al.* 1993). More recent selections from the SJV are probably less sensitive, though data are lacking. Much of the cotton acreage in the SJV is subject to increasing concentrations of O_3.

Ozone affects cotton through direct oxidant damage to physiological processes and ultimately to yield (Grantz, 2003). Direct effects of ozone on photosynthesis (Lehnherr et al., 1987; Pell et al., 1992) do not appear to explain the effects of O_3 on whole plant productivity (e.g. Meyer et al., 1997; Reich and Amundson, 1985; Krupa and Manning, 1988; Rennenberg et al., 1996; Grantz and Yang, 1996; Grantz and Farrar, 1999; McLaughlin and McConathy, 1983) Inhibition of carbohydrate export from source leaves may reduce photosynthesis indirectly while directly impacting carbon allocation and growth. For example, O_3 reduced assimilation in wheat (Meyer et al., 1997), but this was attributed to feedback inhibition of photosynthetic enzymes caused by sucrose accumulation. Simulation studies (Grantz et al., 1999) suggested that inhibited root development (Grantz and Yang, 1996) caused by O_3 may mediate some observed O_3 impacts on shoot function. It is not known if carbohydrate translocation may be a primary target of O_3 inhibition.

Cotton is typical of many plants that translocate mostly sucrose in the phloem, although some raffinose series sugars are present. . It is thought that transport of raffinose series sugars differs from that of sucrose in that phloem loading is symplastic, not involving an apoplastic, transmembrane, step (Bachmann et al., 1994; Kandler, 1967; Dey, 1985; Turgeon and Gowan, 1992). The symplastic mechanism (eg. Madore and Webb, 1981; Turgeon and Gowan, 1992) could afford some protection against oxidant damage. Sucrose uptake was inhibited by the sulfhydryl reagent, PCMBS (p-chloromercuribenzene-sulfonic acid; Madore, 1990). A preliminary comparison of bean (*Phaseolus vulgaris*), a sucrose transporter, with basil (*Ocimum basilicum* L.), a stachyose transporter, demonstrated a lower sensitivity of phloem exudation to O_3 and to PCMBS in basil than in bean (M. Madore; personal communication).

In previous studies under different exposure conditions (Grantz and Yang, unpublished) we found that O_3 inhibited the magnitude of phloem loading in Pima cotton (Grantz and Farrar, 1999) and restricted allocation of newly fixed C to developing roots and fruits (Grantz and Yang, 1996). In previous studies we also found that the ratio of sucrose to raffinose series sugars was reduced dramatically in sink root tissue but unchanged by O3 in source leaf tissue. Here we have used aphid honey dew as a minimally disruptive probe to examine O_3 impacts on the sugars in phloem sap in Pima cotton grown in field exposure chambers. These are compared with sugar profiles in source and sink tissue obtained under the same exposure conditions.

Materials and Methods

Plant Material

Cotton (cv. Pima S6) was grown from seed in 9-l tapered plastic pots (Treepot; Hummert International, Earth City, MO), filled with sintered clay (6-40 mesh; Quicksorb, A & M Products, Taft, CA).

Pots were randomly assigned to outdoor chambers for exposure to one of three concentrations of ozone. Pots were automatically irrigated to run-through up to three times a day as required by the weather. A complete fertilizer (Miracle Gro; Scotts Miracle-Gro Products Inc., Port Washington, NY) was injected into the irrigation water at 1.3 g l^{-1} weekly.

Ozone Exposure

Experiments were conducted in Open Top Chambers (OTCs; 3.1 m diameter x 2.4 m height; Heagle et al., 1973) at the University of California, Kearney Agricultural Center, Parlier, CA (103 msl, 36.598 N 119.503 W). Ozone was generated by corona discharge (Model G22; Pacific Ozone Technology, Brentwood, CA) from oxygen (Model AS-12; AirSep Corporation, Buffalo, NY). The daily timecourse of O_3 concentration was regulated in a single OTC using a dedicated O_3 monitor (Model 49C, Thermo Environmental Instruments, Franklin, MA). This monitor was interfaced to a computer for feedback control, as described previously (Grantz et al., 2003). The low O_3 (LO3) regime was charcoal filtered air (CF) and was nominally O_3-free (actual 12 hour mean, hm = 15.9 ppb). The medium O_3 (MO3) regime approximated the diurnal profile and maximal concentration observed on exceptionally polluted days at this location (actual 12 hm = 80.6 ppb). The high O_3 (HO3) regime was approximately 1.9-fold greater than the MO3 at each time point (actual 12 hm = 153.6 ppb).

Tissue

All measurements were performed on youngest fully expanded leaves or on fine roots. Roots were sampled after the sintered clay potting medium was removed by agitation in cold water. Tissue was quick-frozen in liquid N_2 and stored at -80°C until lyophilized. Lyophilized tissue was diced finely with a razor blade, weighed and transferred to a 1.7 ml screw cap plastic tube to which 1.0 ml water was added, the tube shaken, then placed in a Branson 85 W sonicator filled with ice water for 15 min (Murray, 1998). The extraction was repeated twice using 1.0 and 0.5ml of water respectively.

Phloem Sap

A colony of cotton aphid (*Aphis gossypii*), was established from individuals obtained from the USDA/ARS aphid colony in Parlier, CA. The aphids were reared on young cotton plants in a greenhouse. Aphids were transferred to the field OTCs in small custom designed “clip cages”. These were 3 cm long made from 2.5 cm diameter plastic tubing. Air holes were cut in the sidewall and covered with fine mesh. One end of the tube was closed with a spring loaded clear plastic cover attached to the tube with a large hair clip. The other end of the container was the honeydew collection surface, a piece of aluminum foil formed tightly over the end of the tubing. This was easily removed and replaced during sample collection.

Approximately 10 aphids were transferred to each clip cage using a camel hair brush. Four clip cages were placed in each OTC, attached to the youngest fully expanded leaves of different plants. Containers were supported with a wire frame to maintain orientation with the aluminum foil at the bottom for efficient honeydew collection. Aphids were allowed to adapt to the experimental plants and to purge for 24 hours at which time the aluminum foil was discarded. A new piece of aluminum foil was attached and honeydew collected for 3 days. Containers were washed and dried prior to installation on a plant.

The aluminum foil with deposited honeydew was immediately dried (40 C, 24 hours), then stored in individual Petri dishes sealed with Parafilm, until extraction.

Honeydew was eluted with water, freeze dried and brought to known volume. Carbohydrate analysis was performed by HPAEC-PAD (High pH Anion Exchange Chromatography with Pulsed Amperometric Detection) on a Dionex Bio-LC, with a Dionex CarboPac PA 1 column. The eluent was 150mM NaOH, isocratic for 5 minutes, followed by a linear sodium acetate gradient from 0 to 500mM in 150mM NaOH, for 35 minutes . Retention times are expressed in minutes and detector response in µCoulombs (see Murray et al., 1997, 1999 in these Beltwide Proceedings, for further details).

Chromatographic analysis of honeydew and tissue extracts was performed with Dionex PeakNet software, transferred to an Excel spreadsheet. For known sugars, for which authentic standards were available, standard curves based on peak areas were used. Peak areas or sugar amounts were analyzed for O_3 effects over all samples using a 1-way ANOVA (PROC GLM; SAS Institute, 1990).

Results and Discussion

Growth

Growth of cotton plants was negatively impacted by exposure to ozone. Plants were significantly shorter (Grantz and Shrestha, this volume) and produced significantly fewer leaves and less biomass with increasing ozone.

Sugar composition

Source. Source leaf tissue of Pima S-6 grown in field exposure chambers exhibited a ratio of sucrose to raffinose series sugars (sum of raffinose, stachyose and verbascose) slightly in excess of 3:1 in LO3 (Figure 1B) This declined in a dose dependent fashion, but only slightly with increasing ozone exposure.

Sink. The content of sucrose in fine root sink tissue declined substantially from LO3 to MO3 and HO3 (Fig. 1A, squares). This ratio declined from about 10:1 to about 6:1, with no further change as ozone increased from MO3 to HO3.

These data, consistent with our previous results (Grantz and Yang, unpublished) demonstrate that sugar profiles in sink and source tissues are uncoupled. The sugar profiles observed in sink tissues represent whole tissue extractions, and may reflect either the composition of the phloem sap feeding the sink tissue, or a consequence of metabolism during transport or in the sink. It was important to examine the composition of the phloem sap, which links the two tissues.

Phloem sap. Cotton is generally considered to translocate primarily sucrose in the phloem sap. However, it is clear from our data and that of others (Henneberry et al., 2000) that this is an oversimplification. Both sucrose and raffinose series sugars (raffinose, stachyose, verbascose, ajugose and members of greater degree of polymerization (including putative DP-7 and DP-8) are present in the honeydew (Figure 2). DP-7 is the peak appearing just prior to DP-8 (Fig. 2). The chromatograms clearly resolve a large number of known and unknown sugars. Some, particularly the larger species, may be artefacts of honeydew collection and not representative of phloem sap. However, sugar composition of the sap (analyzed as honeydew) responded substantially to ozone exposure (Fig. 1A, circles).

The relative abundance of sucrose declined with increasing O_3 concentration, while the relative content of raffinose and stachyose, as well as DP-7, increased. In this pair of contrasting samples (Figure 1) the relative abundance of sucrose declined only modestly as the O_3 concentration increased. Over all samples (n = 104) the relative abundance of sucrose declined by approximately 18% while that of the known raffinose series sugars increased by 17%.

This resulted in a substantial decline in the average ratio of the identified sugars, sucrose:raffinose series (Figure 2). These data agree well with the sink tissue constituents obtained previously with the same cultivar (above) and not with profiles obtained from source leaf tissue.

The tentative conclusion from these data, and a basis for more detailed experiments, is that the sugar profiles of the sink tissues reflects that of the phloem sap that is unloaded in the sink, rather than that of the source from which the phloem is loaded. The relative reduction in sucrose and increase in raffinose series sugars could implicate the apoplastic step of sucrose loading as a more sensitive site of O_3 attack than the symplastic phloem loading processes of raffinose series sugars.

The data are preliminary in the sense that aphid honeydew is modified relative to its source, the actual phloem sap. Further experiments are planned with cotton and other species to characterize the extent of the modification.

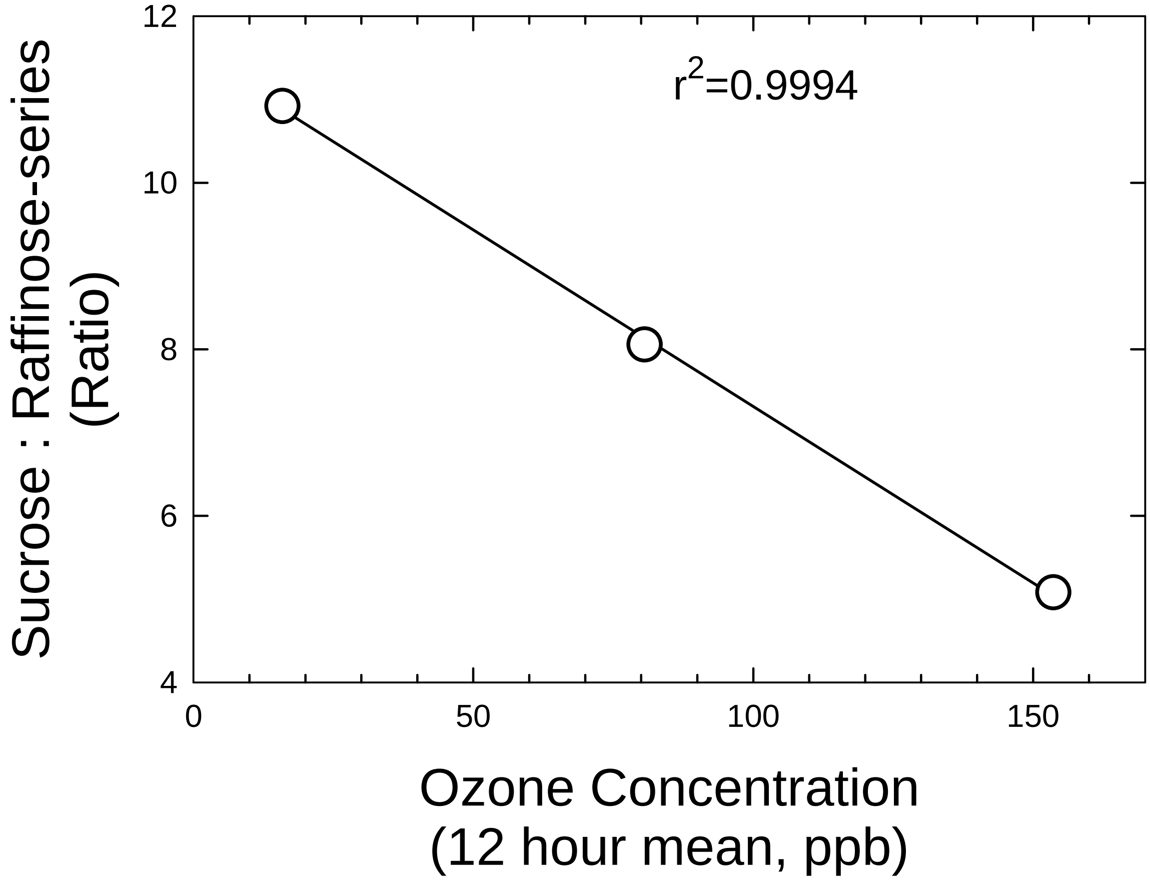

Figure 1. Effect of increasing O_3 exposure on the ratio of sucrose to the principal known raffinose series sugars (Sum of Raffinose, Stachyose and Verbascose) in phloem sap sampled as aphid honeydew from Pima S-6 plants.

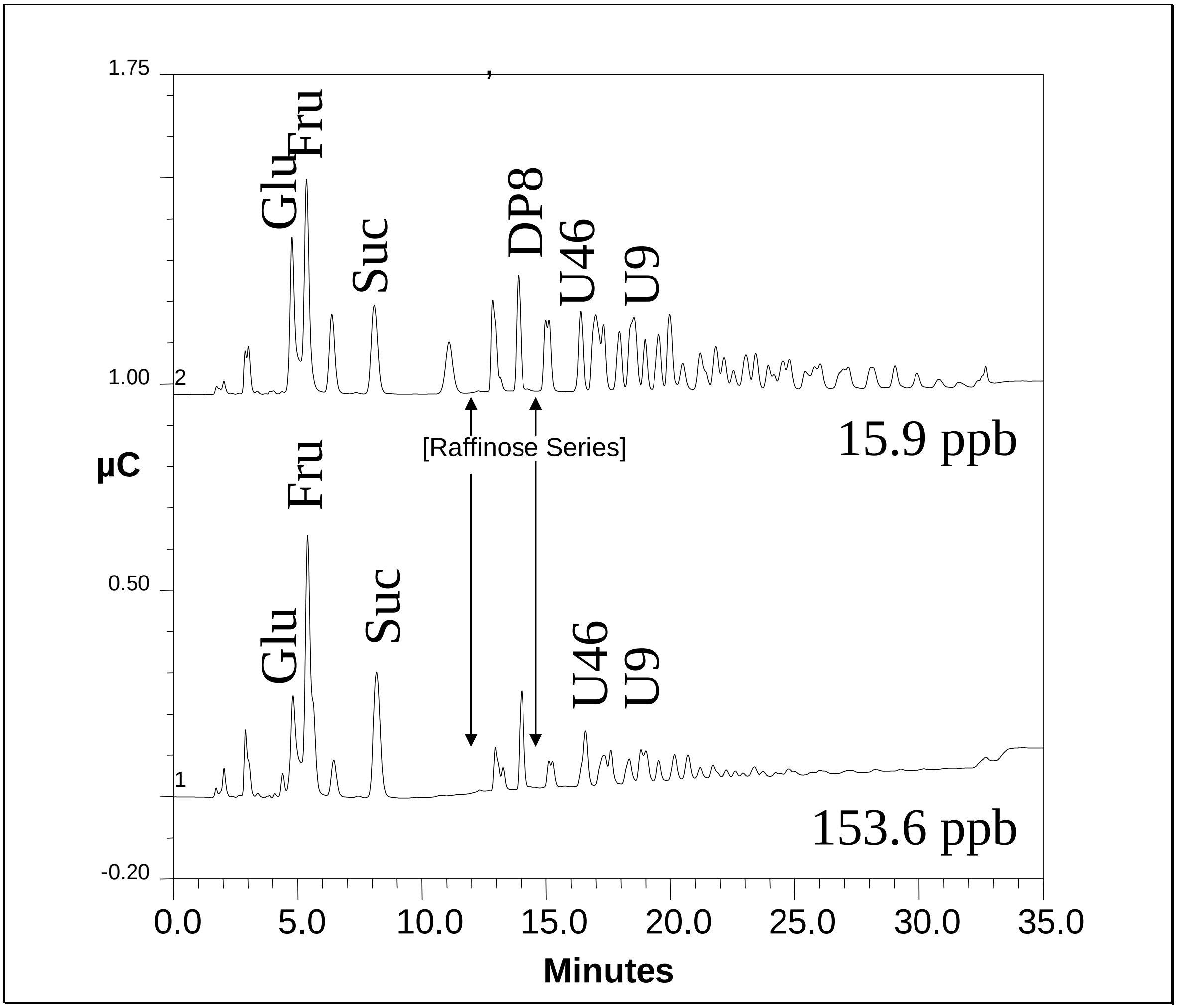

Figure 2. Representative chromatograms resolving a large number of known and unknown sugar constituents in phloem sap sampled as aphid honeydew from Pima S-6 plants exposed to low (top) and high (bottom) concentrations of O_3.

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CARBOHYDRATE COMPOSITION OF COTTON APHID HONEYDEW

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Abstract

Honeydew from cotton aphids (*Aphis gossypii)* has been collected from aphids feeding on Pima S-6 plants between the ages of 4 and 7 weeks. The sugars identified in the honeydew are the same as those identified from honeydew of aphids feeding on older plants of upland cottons with the exception that we did not find trehalulose and melizitose. The sucrosyl oligosaccharides and many larger oligosaccharides were also found. The quantity and and distribution of the larger oligosaccharides varies with the age of the plants with the greatest concentration being present at five and six weeks of age. The larger oligosaccharides are not reducing sugars consistent with their proposed biosynthesis from sucrose and sucrosyl oligosaccharides.

Introduction

The sugar composition of cotton aphid honeydew which feed on cotton phloem sap has been reported to contain a number of sugars and polyols (Hendrix, 1999, Henneberry, et. Al, 2000). Honeydew has been shown to contain several monosaccharides, disaccharides and a large number of glucose containing oligosaccharides which have not been identified. These studies have been done on the honeydew of aphids feeding on mature plants and the interest has been with respect to sticky cotton. The present work is part of a larger investigation however we report here on the sugar composition of cotton aphid honeydew and the differences in the sugar composition with young plants of different ages.

Materials and Methods

Cotton (cv. Pima S6) was grown from seed in 9-l tapered plastic pots (Treepot; Hummert International, Earth City, MO), filled with sintered clay (6-40 mesh; Quicksorb, A & M Products, Taft, CA).
Pots were randomly assigned to outdoor chambers for exposure to one of three concentrations of ozone. Pots were automatically irrigated to run-through up to three times a day as required by the weather. A complete fertilizer (Miracle Gro; Scotts

Miracle-Gro Products Inc., Port Washington, NY) was injected into the irrigation water at 1.3 g l^{-1} weekly.

A colony of cotton aphid (*Aphis gossypii*), was established from individuals obtained from the USDA/ARS aphid colony in Parlier, CA. The aphids were reared on young cotton plants in a greenhouse. Aphids were transferred to the field OTCs in small custom designed “clip cages”. These were 3 cm long made from 2.5 cm diameter plastic tubing. Air holes were cut in the sidewall and covered with fine mesh. One end of the tube was closed with a spring loaded clear plastic cover attached to the tube with a large hair clip. The other end of the container was the honeydew collection surface, a piece of aluminum foil formed tightly over the end of the tubing. This was easily removed and replaced during sample collection.

Approximately 10 aphids were transferred to each clip cage using a camel hair brush. Four clip cages were placed in each OTC, attached to the youngest fully expanded leaves of different plants. Containers were supported with a wire frame to maintain orientation with the aluminum foil at the bottom for efficient honeydew collection. Aphids were allowed to adapt to the experimental plants and to purge for 24 hours at which time the aluminum foil was discarded. A new piece of aluminum foil was attached and honeydew collected for 3 days. Containers were washed and dried prior to installation on a plant. The aluminum foil with deposited honeydew was immediately dried (40 C, 24 hours), then stored in individual Petri dishes sealed with Parafilm, until extraction.

Honeydew was eluted with water, taken to dryness in a SpeedVac and brought to known volume. Carbohydrate analysis was performed by HPAEC-PAD (High pH Anion Exchange Chromatography with Pulsed Amperometric Detection) on a Dionex Bio-LC, with a Dionex CarboPac PA 1 column. The eluent was 150mM NaOH, isocratic for 5 minutes, followed by a linear sodium acetate gradient from 0 to 500mM in 150mM NaOH, for 35 minutes . Retention times are expressed in minutes and detector response in µCoulombs (see Murray et al., 1997, 1999 in these Beltwide Proceedings, for further details). Chromatographic analysis of honeydew was performed with Dionex PeakNet software, transferred to an Excel spreadsheet. For known sugars, for which authentic standards were available, standard curves based on peak areas were used. peak areas for sugar amounts. Cellobiose was used as the internal standard.

Results

The honeydew samples were highly variable in their sugar content but there were consistent patterns of the distribution of sugars. The samples all contained fucose, arabinose, glucose, fructose and sucrose. The presence of larger oligosaccharides, including the sucrosyl oligosaccharides which consist of sucrose with increasing galactose residues on positon 6, raffinose, stachyose, verbascose, ajugose and the next two sugars in the series of sucrosyl oligosaccharides the heptasaccharide and octasaccharide was dependent on the age of the plants. A comparison of the chromatographic profiles of the sugars in honeydew from plants aged 4, 5, 6 and 7 weeks is shown in Figure 1. The presence of at least 30 larger oligosaccharides is clearly shown in the honeydew from plants aged five and six weeks. A lesser amount of these larger oligosaccharides is present in the honeydew from 4 week old plants but they were almost completely absent from honeydew from seven week old plants.

Discussion

The carbohydrate composition of cotton aphid honeydew reported here is very similar to that reported by Hendrix (Hendrix, 1999) with the major difference being the absence of trehalulose and melizitose in these samples. The presence of large oligosaccharides was also reported by Hendrix. In this report we employed a more shallow sloped salt gradient to resolve the larger oligosaccharides. The earlier work was focused on the role of honeydew from older plants and its role in sticky cotton. In this case, the honeydew was collected from much younger plants as part of a larger study. We did not anticipate the observed difference in honeydew composition with plants of different ages. These differences may reflect differences in phloem constituents with developmental age of the plants. At this time it is unclear which of the larger oligosaccharides may be the result of polymerization of sucrose by the aphids and which of the oligosaccharides may arise from the polymerization of oligosaccharides larger than sucrose in the phloem. Most of the larger oligosaccharides are not reducing sugars.

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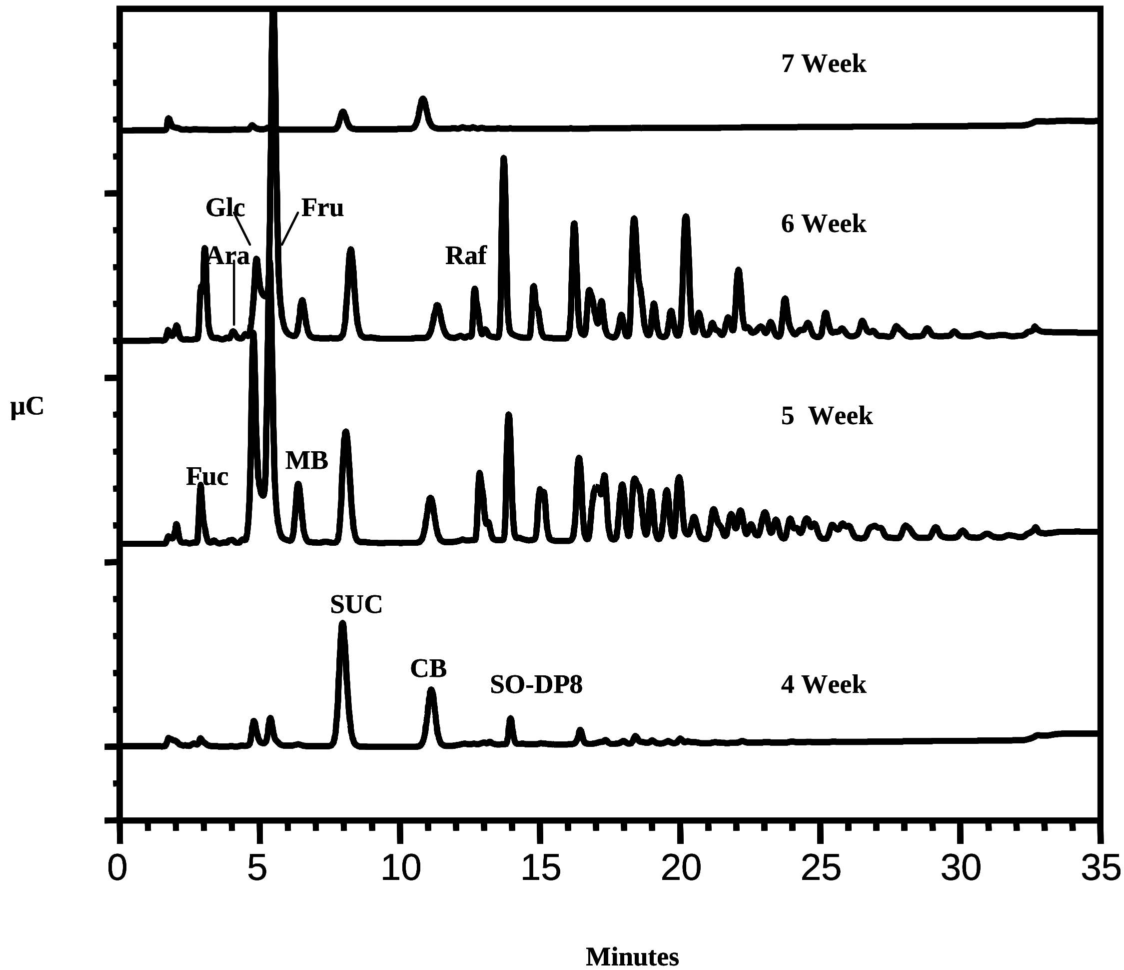

Figure 1. Representative chromatograms demonstrating the presence of known and unknown carbohydrates in honeydew from cotton aphids feeding on Pima S-6 plants at ages 4, 5, 6 and 7 weeks. Abbreviations: Ara:arabinose, Glc:glucose, Fru:fructose, MB:melibiose, Suc:sucrose, CB:cellobiose(internal standard), Raf:raffinose and SO-DP8:sucrosyl oligosaccharide degree of polymerization 8.

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Journal of Experimental Botany

RESEARCH PAPER

Ozone increases root respiration but decreases leaf CO_2 assimilation in cotton and melon

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Abstract

It is well established that exposure of plant foliage to tropospheric ozone (O_3) inhibits photosynthetic gas exchange in leaves and the translocation of current photosynthate to sink tissues. It is less clear what impact O_3-reduced source strength has on the physiological responses of sink tissue such as fine roots. The responses were investigated of carbon acquisition in leaves and carbon utilization in the respiration of fine roots, following chronic (weeks) and acute (hours) exposures to O_3 in open top chambers. Previous reports indicate increased, decreased, and unchanged rates of root respiration following exposure to O_3. A decline in source activity is confirmed, but an increase in sink respiration is reported in fine roots of Pima cotton (cv. S-6) and muskmelon (cv. Ambrosia hybrid). Leaf source strength and root sink activity changed in opposing directions, thus there was no positive correlation that might indicate direct substrate control of root function. Additional linkages between shoot and root following exposure to O_3 may be involved.

Key words: Allocation, cotton, *Cucumis melo*, gas exchange, *Gossypium barbadense*, melon, ozone, photosynthesis, root respiration.

Introduction

Carbon acquisition and allocation

Chronic exposure to O_3 inhibits allocation of biomass to developing roots in Pima cotton (*Gossypium barbadense* L.; Grantz and Yang, 1996), muskmelon (*Cucumis melo* L.; Fernandez-Bayon *et al.*, 1993; DA Grantz and S Yang, unpublished data), and many other species (Cooley and Manning, 1987; Reiling and Davison, 1992; Darrall, 1989; Rennenberg *et al.*, 1996). In cotton (Grantz and Yang, 1996, 2000) the root/shoot biomass ratio decreased and leaf area specific root hydraulic conductance declined despite reduction of leaf area. Degraded root system function may contribute to O_3-induced inhibition of shoot gas exchange and carbon acquisition (Grantz *et al.*, 1999).

Numerous studies have demonstrated an O_3-induced reduction in photosynthetic carbon assimilation (A_n; Reich, 1983; Dann and Pell, 1989; Farage *et al.*, 1991) in Pima cotton (Grantz and Farrar, 1999, 2000), muskmelon (Fernandez-Bayon *et al.*, 1993) and other cucurbits (Castagna *et al.*, 2001; Fernandez-Bayon *et al.*, 1993). A parallel reduction of stomatal conductance (g_s) was often observed, confirming, and in some cases contributing, to the observed limitation of A_n. Both direct and indirect impacts of O_3 on A_n reduce the quantity of carbohydrate (CHO) available for export from source leaves to sink tissues such as fine roots. In addition, the inhibition of export of recent photosynthate may further limit CHO supply to roots (Grantz and Farrar, 1999, 2000; Darrall, 1989).

Root respiration

The reduced allocation of CHO to roots must eventually reduce substrate availability for root growth and maintenance respiration. In tomato, O_3 reduced substrate availability and the production of root exudates (McCool and Menge, 1983) which adversely affected mycorrhizal infection. O_3-induced increases in the translocation of photosynthate to roots have also been observed, particularly with low O_3 concentrations (Ponderosa pine: Scagel

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Abbreviations: A_n, net carbon assimilation; R_r, fine root respiration; g_s, stomatal conductance; OTC, open top exposure chamber; CF, charcoal-filtered air; MO3, HO3, medium or high ozone concentration; CHO, carbohydrate; Q, respiratory quotient.

and Andersen, 1997; *Trifolium repens*: Blum *et al.*, 1983; *Triticum aestivum:* McCrady and Andersen, 2000).

Responses of fine root respiration (R_r) to O_3 remain unclear, with previous studies providing an array of contrasting conclusions. O_3 decreased R_r in some conifers (*Pinus taeda:* Edwards, 1991; *P. armandi:* Shan *et al.*, 1996; *Pseudotsuga menziesii:* Gorisson and van Veen, 1988), and in such annual crops as *Phaseolus vulgaris* (Hofstra *et al.*, 1981; Ito *et al.*, 1985). By contrast, O_3 increased R_r in other conifers (*Pinus ponderosa*: Scagel and Andersen, 1997) and temperate broad-leafed trees such as deciduous red oak (*Quercus rubra*: Kelting *et al.*, 1995). The extent to which these conflicting observations reflect interspecific variability, contrasting experimental conditions, or inherent variability in measured values of R_r, remains unknown. These studies report chronic O_3 exposures which may have allowed the acclimation of allometry, root system morphology, and physiological function. Such a restoration of homeostasis confounds the interpretation of primary O_3 impacts on R_r.

As R_r may consume over half of the net primary productivity (Lambers *et al.*, 1996) and up to 75% of CHO translocated to roots (Högberg *et al.*, 2002), the magnitude and direction of O_3 impacts on this below-ground carbon sink are of considerable interest. The diversity of O_3 effects on R_r noted above has hindered the prediction of O_3 impacts on carbon sequestration as part of the overall effects of global change, and has prevented appropriate parameterization of long-term O_3 impacts on plant growth and development. O_3 effects on R_r could also serve as early diagnostic signals of O_3 damage to vegetation (Richards, 1989; Taylor and Ferris, 1996).

Current investigation

O_3 impacts on cotton and melon, species which use different mechanisms of phloem loading and contrasting primary transport sugars, were used to test three hypotheses: (1) chronic (long-term) exposure to O_3 reduces R_r; (2) chronic changes in R_r are correlated with changes in A_n; (3) acute (short-term) exposure to O_3 reduces R_r and A_n in parallel or sequentially.

Hypotheses 1 and 2 are rejected, as chronic O_3 exposure significantly increased R_r in both cotton and melon, while reducing leaf A_n. Over short exposures, the O_3 effects on R_r were more variable and hence more difficult to identify, despite a high degree of replication. The acute effects were entirely consistent with chronic impacts, though temporal resolution was inadequate to evaluate Hypothesis 3 in a definitive manner. Leaf source strength and root sink activity changed in opposing directions over all time scales considered, with no suggestion of a positive correlation indicative of direct substrate control of R_r. Additional and previously unappreciated linkages between shoot and root may be involved in O_3 phytotoxicity.

Materials and methods

O_3 and environmental exposure

The Open Top Chamber (OTC; 3.1 m diameter × 2.4 m height; Heagle *et al.*, 1973) facility at the University of California Kearney Agricultural Center (103 m elevation, 36.598 N 119.503 W) was used for all experiments. Ten OTCs are available at this facility, with nine used in the present studies.

O_3 was generated by corona discharge (Model G22; Pacific Ozone Technology, Brentwood, CA) from oxygen (Model AS-12; AirSep Corporation, Buffalo, NY). The daily time-course of O_3 concentration was regulated in a single OTC using a dedicated O_3 monitor (Model 49C, Thermo Environmental Instruments, Franklin, MA) interfaced to a computer for feedback control. The other eight OTCs were controlled using manual proportioning valves to control the flow of O_3. O_3 concentration was determined in all OTCs approximately every 15 min. Air was sampled continuously from the centre of each chamber through a Teflon dust filter and teflon tubing attached to a multiport solenoid valve. All O_3 concentration data were archived electronically.

Three O_3 exposure regimes were imposed. The charcoal-filtered treatment (CF) was nominally O_3-free (Table 1), but exhibited slightly positive O_3 concentrations, nearly equivalent to global (pristine) background concentrations. This occurred due to imperfections in the charcoal filters and incursion of ambient air through the open top of each OTC, despite the installation of a conical frustrum. The medium O_3 treatment (MO3) reproduced the diurnal profile and maximal concentration observed on exceptionally polluted midsummer days at this location (Table 1). The high O_3 treatment (HO3) was 1.6-fold greater than MO3 at each time point. The O_3 concentrations achieved during the acute, short-term exposures are summarized as accumulated concentration with no threshold (SUM 00; Table 2) and as means of all (day and night) concentrations (Mean; Table 2). The exposures were the same each day.

Chronic exposure experiment

Seeds of cotton (*Gossypium barbadense* L. cv. Pima S-6; JG Boswell Co., Corcoran CA) or melon (canteloupe/muskmelon; *Cucumis melo* cv. Ambrosia Hybrid; Burpee Seed Co., Warminster, PA) were sown approximately 2 cm deep in 6–40 mesh sintered clay (QuickSorb, A&M Products, Taft, CA) in 9.0 l tapered (45 cm deep, 18 cm diameter) pots (Treepot; Hummert International, Earth City, MO). Pots were washed with water prior to planting. Pots containing ungerminated seed were irrigated to run-through and randomized among the three O_3 exposure regimes. Seedlings were thinned to one per pot as the first true leaf began to expand.

Plants were grown for approximately 6 weeks (cotton) or 5 weeks (melon) until they had attained five leaves. A total of seven (cotton) or 10 (melon) independent experiments, each with at least two plants per treatment, were conducted. Experiments were randomly assigned to the replicate CF, MO3 and HO3 OTCs. Pots were automatically irrigated to run-through at least daily and up to several times per day as required by the weather. A complete fertilizer

Table 1. *Nominal exposure characteristics for three ozone exposure regimes (ppb)*

Ozone treatment	Daily maximum	12 h mean
CF	0	0
MO3	140	90
HO3	224	143

Table 2. *Ozone exposure characteristics (sum with no threshold, mean for all hours including night) for the sample periods in the acute O_3 exposure regimes*

Ozone treatment	Exposure duration	Sum 00 (ppb h) (Mean±SE)	24 h mean (ppb) (Mean±SE)
CF	1 h	8.23±1.48	8.23±1.48
CF	3 h	29.6±4.2	9.86±1.40
CF	5 h	48.6±7.4	9.71±1.49
CF	29 h	298±56	10.3±2.0
CF	53 h	545±139	10.3±2.6
HO3	1 h	142±12	142±12
HO3	3 h	475±28	158±10
HO3	5 h	944±64	189±13
HO3	29 h	3602±382	124±13
HO3	53 h	6611±827	125±16

(Miracle Gro; Scotts Miracle-Gro Products Inc., Port Washington, NY; 1.3 g l^{-1}) was applied to run-through weekly.

Data were analysed by 2-way ANOVA, with measurements classified by O_3 treatment and date of measurement. The two species were analysed separately.

Acute exposure experiment

Seeds of cotton and melon were planted in individual conical tubes (Ray Leach Single Cell Cone-Tainers; 4 cm diameter × 20 cm deep; Hummert International, Earth City, MO). Seeds were planted approximately 2 cm deep in 6-40 mesh sintered clay (QuickSorb). 35 tubes were spaced evenly in a cone tray (Hummert International, Earth City, MO). Tubes were sterilized with hypochlorite (10%; 3 min) and rinsed prior to planting.

Germination and growth were in a heated, whitewashed greenhouse. Each container was irrigated to run-through daily, twice daily as required by the weather, and fertilized weekly (Miracle-Gro; 3.1 g l^{-1}). Plants were grown for approximately 3 weeks until they had attained two fully expanded true leaves during the period 20 March–23 October, 2002. 48 h before experiments cotyledons were excised to limit carbohydrate supply to root tissue from stored photosynthate. Experiments were initiated at 09.00 h PDT when plants were transferred from the greenhouse to a CF OTC. Plants in OTCs were irrigated every 2 h with automated drip irrigation.

Plants were allowed to acclimate to the modified environment for 1 h. At 10.00 h exposure timing began when a randomly selected 50% of plants were transferred to a HO3 OTC, leaving 50% of plants in the CF OTC. Measurements on cotton and melon were conducted on different, often alternating, days, at 1, 3, 5, 29, and 53 h after initiation of O_3 exposure. Mean and accumulated O_3 exposures for each treatment are shown in Table 2. A total of 19 independent experiments were performed with cotton and 15 independent experiments with melon, each with at least two plants per treatment. All short-term exposures were conducted in the same CF, MO3 and HO3 chambers.

Data were analysed by paired sample *t*-tests (matched CF, HO3 samples) within individual exposure times (1–53 h) and over all measurements taken together. No consistent trends were observed over the time of exposure nor date of measurement. The two species were analysed separately

Gas exchange

Measurements of A_n and g_s were obtained on the youngest fully expanded leaf, using a steady-state gas exchange system (Model LI-6400; Li-Cor Inc., Lincoln, NE). The LI-6400 was operated with an internal light source (1000 μmol m^{-2} s^{-1}; blue (20%) and red (80%) light emitting diodes; Li-Cor) and with control of CO_2 concentration in the cuvette (C_a; 400 ppm), using small cylinders of pressurized CO_2, and a constant flow (500 μmol s^{-1}). A_n and g_s were expressed relative to projected leaf area of the measured leaf.

Root respiration

Intact root systems were obtained from two plants in each treatment at each measurement time point. The container and the sintered clay potting medium were removed by immersion in cold water. The medium was removed (virtually quantitatively) from the roots by gentle agitation. The terminal 3–4 cm of fine root were excised and immediately transferred to a respirometer chamber.

Fine root respiration (R_r) was determined in liquid phase with a Clark-type oxygen electrode (Delieu and Walker, 1972). Four respirometer chambers (Oxygraph Oxygen Electrode System; PP Systems, Haverhill, MA) were run in parallel, interfaced with a computer for data acquisition and analysis. A magnetic stir bar was placed in each chamber, separated from the root material by a porous metal screen. Temperature control (25 °C) was maintained by the circulation of water through a precision water bath (Model 9100, Isotemp Pittsburgh, PA,) and through the plastic housing of each respirometer chamber.

Electrodes were calibrated using air-saturated water, and oxygen-free water obtained by adding a small amount of sodium dithionite to each chamber. Following several rinsings, 2 ml of water was placed in each chamber. When output had become stable (about 10 min), fine root samples were introduced. R_r was expressed relative to the blotted wet mass of root in each chamber.

Dark/sunlit test of root respirometer measurements

To confirm that differences in R_r would be detected using these methods, two independent experiments were performed with sunlit and dark-adapted cotton. Plants were grown in the greenhouse in Cone-Tainers as for acute exposure experiments, and acclimated for 1 d outside, sheltered from direct sun and the night sky. In the early morning, plants were irrigated to run-through, randomly assigned to two groups, and placed either in an open location and irrigated hourly (sunlit) or shrouded in dark plastic and placed in a deeply shaded, outdoor location (dark adapted). R_r was determined, as above, in mid-afternoon (15.00 h PDT). The experiments did not differ so data were pooled and analysed by unpaired *t*-test with 12 individual plants per treatment.

Results and discussion

O_3 exposure

Experiments were conducted under field exposure conditions, and over the range of environmental conditions observed during the commercial growing season for both cotton and melon in the San Joaquin Valley. Temperature and cloud cover were more variable early and late in the season than during midseason (not shown). Solar radiation was relatively constant from day to day in this semi-arid environment, although day length and maximum insolation changed seasonally (Fig. 1A). The same O_3 profile was imposed on all days, at three concentrations defined as CF, MO3, and HO3 (above). Mean time-courses and variability attained in the CF and HO3 treatments are shown in Fig. 1B. The modest seasonal shifts in alignment of instantaneous O_3 concentration (Fig. 1B), and environmental parameters such as solar radiation (Fig. 1A) were

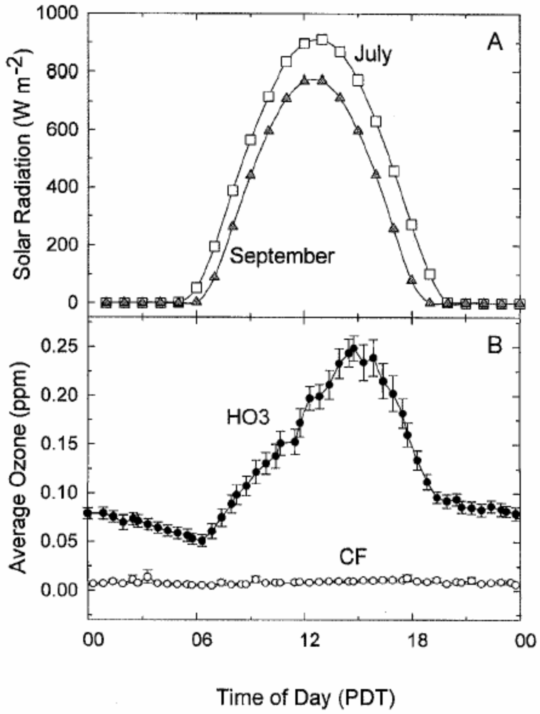

Fig. 1. Representative daily time-courses of (A) solar radiation measured above the OTCs during mid- (squares) and late- (triangles) growing season, 2002; and (B) representative O_3 concentrations (mean $\pm$SE over all initial days of acute exposure experiment in O_3-enriched (solid line and filled symbols) and charcoal-filtered (broken line and open symbols) open top chambers.

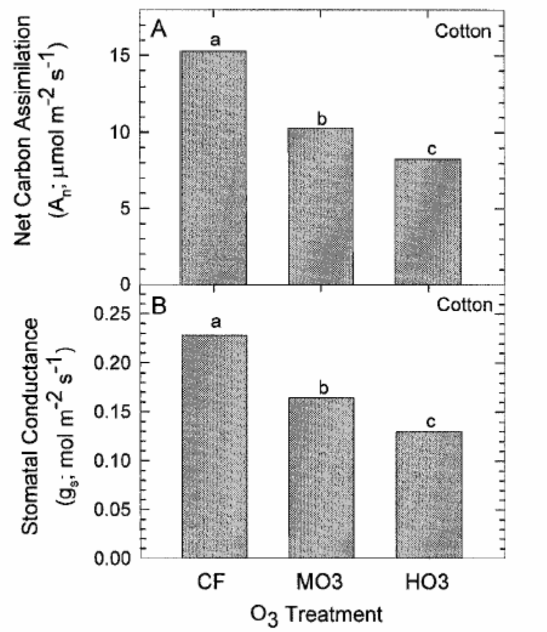

Fig. 2. Effect of chronic exposure to contrasting O_3 concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of cotton. Mean $\pm$SE. Bars with different letters are different at P <0.05.

not associated with seasonal trends in physiological parameters nor sensitivity to O_3. The range of conditions introduced some variability, but increased the generality of conclusions to be drawn from these observations.

Leaf gas exchange

Leaf gas exchange was adversely affected by chronic exposure to O_3. In the youngest fully expanded leaves of both cotton (Fig. 2) and melon (Fig. 3), mid-afternoon values of photosynthetic carbon assimilation (A_n; Figs 2A, 3A) were suppressed by O_3 exposure. The greatest impact was observed between the CF and MO3 treatments, with little further inhibition as the O_3 concentration increased 1.6-fold (Figs 2, 3; cf. MO3, HO3). These results mostly confirmed much of the previous research (Reich, 1983; Dann and Pell, 1989; Farage *et al.*, 1991). The mechanism of photosynthetic inhibition is unclear, and multiple sites of primary oxidant attack are possible, in addition to secondary effects possibly involving water relations (Grantz *et al.*, 1999) and end product inhibition (Darrall, 1989; Goldschmidt and Huber, 1992; Grantz and Farrar, 2000).

A_n was lower in cotton than in melon under these conditions, and considerably more sensitive to O_3 (>30% reduction at MO3 in cotton compared with <25% in melon). The inhibition of A_n per unit area of young, highly productive, leaves underestimates the actual impact of O_3 on whole plant productivity. Carbon assimilation of plants of both species (not shown) was further reduced by O_3-inhibited leaf area production and accelerated senescence and abscission of lower leaves.

Stomatal conductance (g_s) was also reduced by chronic exposure to O_3 in both cotton and melon (Figs 2B, 3B). O_3 often induces parallel declines in g_s and A_n (Grantz and Yang, 1996). The magnitude of inhibition of g_s in the present experiments was similar to that of A_n in cotton, but considerably greater than that of A_n in melon. This stomatal sensitivity may reflect the greater baseline level of g_s in melon than in cotton, although this larger leaf conductance for O_3 entry did not lead to increased sensitivity to O_3 of other processes (e.g. visible symptoms or A_n). The consistency of mesophyll and stomatal responses to chronic O_3 exposure, and the realistic values of calculated intercellular CO_2 concentration (not shown), provide additional support to the accuracy of the measurements of A_n and responses to O_3 under these experimental conditions.

The acute exposure experiments, in which plants were transfered from O_3-free growth conditions to HO3 revealed that the onset of these photosynthetic responses to chronic O_3 exposure was relatively rapid. The time-course data were somewhat confounded by a steady

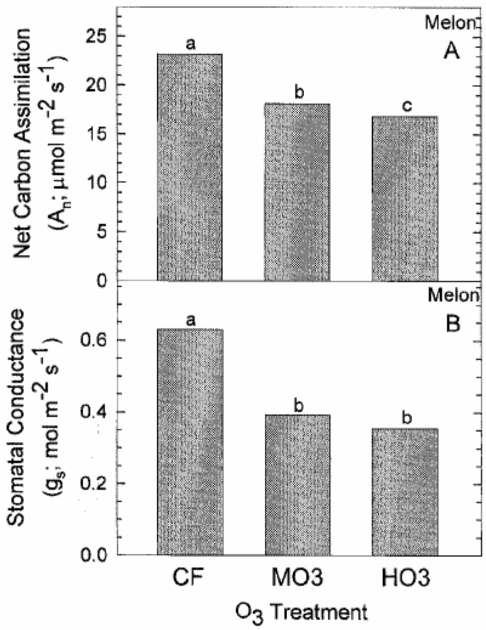

Fig. 3. Effect of chronic exposure to contrasting O_3 concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of melon. Mean ±SE. Bars with different letters are different at P <0.05.

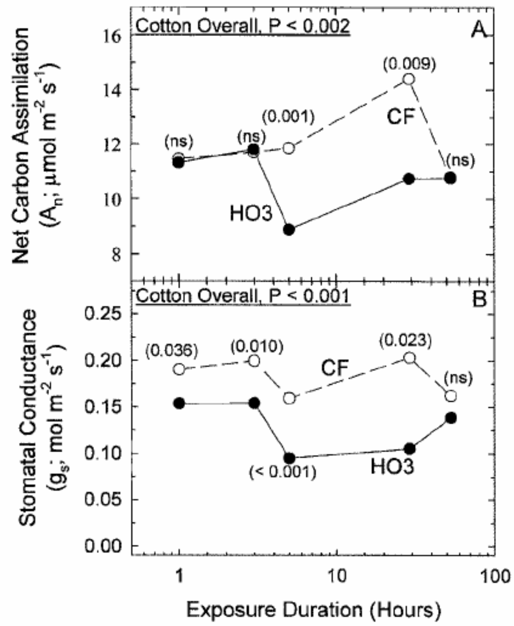

Fig. 4. Effect of acute (short-term) exposure to contrasting O_3 concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of cotton. Mean ±SE. Note log scale for time axis.

increase in A_n in the CF treatment over the first 29 h in cotton (Fig. 4A, open circles) and 5 h in melon (Fig. 5A). Under these conditions (i.e. transfer from one O_3-free environment to another), A_n increased with increasing solar radiation from the first measurement at 11.00 h. A_n may also have undergone acclimation to the higher radiation OTC environment, as suggested by the increase of A_n of CF-treated cotton through mid-afternoon of day 2, before declining precipitously at mid-afternoon of day 3 (53 h; Fig. 4A). A_n of CF-treated melon increased throughout day 1, but declined sharply at mid-afternoon of day 2 (Fig. 5A), before recovering somewhat on day 3. These temporal trends were consistent across many experiments under a range of seasonally variable conditions, but were not further investigated.

A_n of cotton was not inhibited by HO3 during the first 3 h of exposure (Fig. 2A, solid circles), increasing in parallel with the CF-treated plants. By 5 h (15.00 h PDT), a period of high insolation (Fig. 1A) and high O_3 concentration (Fig. 1B), the HO3 plants exhibited a substantial decline in A_n that was not evident in CF-treated plants, resulting in a highly significant difference between the O_3 treatments that was maintained throughout day 2 (29 h). HO3-treated plants remained depressed at mid-afternoon of day 3, but the unexpected decline in A_n of CF-treated plants eliminated significant differences.

In acutely exposed cotton, A_n of CF-treated plants was consistently greater than or equal to A_n of HO3-treated plants. As no consistent diel nor seasonal patterns were observed, data were pooled for analysis over all times and dates. Paired sample t-test of O_3 effects indicated a significant reduction of A_n due to O_3 exposure (P <0.002; Fig. 4A), consistent with observations of the chronically exposed plants described above (Fig. 2A).

The time-courses of A_n in CF- and in HO3-treated cotton were reflected in those of stomatal conductance (g_s; Fig. 4B). Responses of g_s may have preceded those of A_n, This would be an important result, but the data for cotton are insufficient to allow confidence in this conclusion and this was clearly not the case in melon (below). In cotton, g_s was significantly depressed by O_3 within the first hours of exposure, and remained consistently and generally significantly depressed below CF values throughout the 53 h exposure. By mid-afternoon of day 3 (53 h), g_s in the CF-treated plants declined along with A_n, obscuring potential treatment differences. Over all observations, however, O_3 significantly reduced g_s in acutely exposed cotton (P <0.001; Fig. 4B).

In acutely exposed melon, A_n was reduced by O_3 at the first observation following 1 h of exposure, and significantly suppressed at each subsequent timepoint except on day 2 (29 h) when a decline in CF along with HO3 values obscured treatment differences (Fig. 5A). On day 3 (53 h), both CF and HO3 plants recovered, though HO3 remained significantly depressed by O_3. Analysis of all measure-

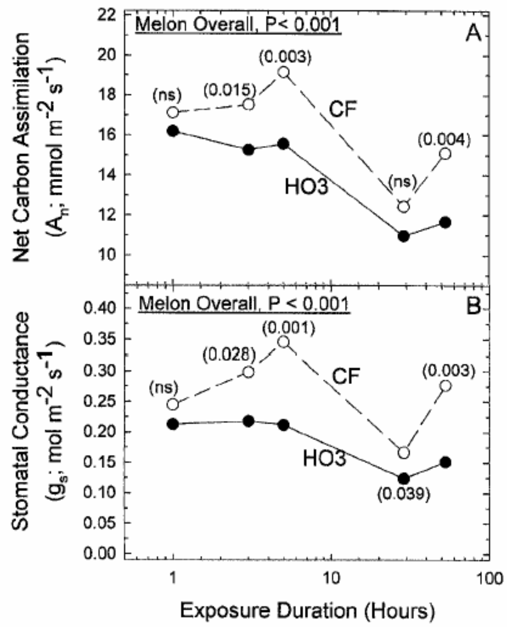

Fig. 5. Effect of acute (short-term) exposure to contrasting O_3 concentrations on gas exchange parameters (A, net carbon assimilation; B, stomatal conductance) of melon. Mean ±SE. Note log scale for time axis.

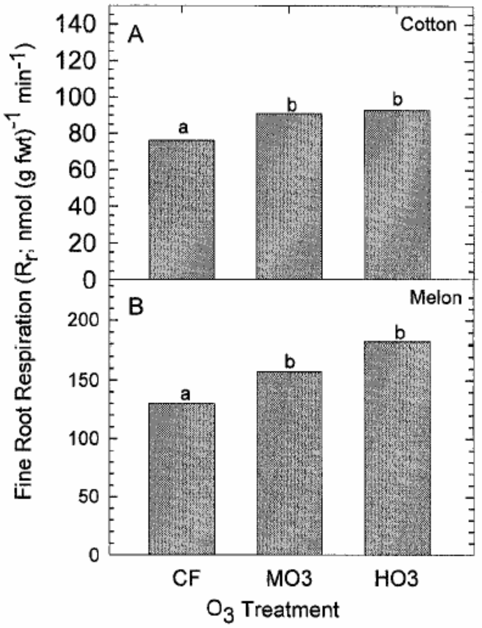

Fig. 6. Effect of chronic exposure to contrasting O_3 concentrations on respiration of fine roots on fresh weight basis (A, cotton; B, melon). Mean ±SE. Bars with different letters are different at P <0.05.

ments demonstrated a significant reduction of A_n by O_3 in acutely exposed melon (P <0.001; Fig. 5A).

A consistent depression of g_s in melon was observed in HO3 relative to CF plants. The impact of O_3 was first observed in parallel with changes in A_n, with a modest depression observed after 1 h of exposure and significant suppression at all subsequent measurements. The depression of gas exchange on the second day of exposure, observed in both g_s and A_n, differed from observations in cotton (cf. Figs 4, 5) in which maximum values of A_n and g_s were observed at this time. O_3 significantly depressed g_s of acutely exposed melon over the entire experiment (P <0.001; Fig. 5B).

Root respiration

Fine root respiration (R_r) was significantly affected by chronic exposure to O_3. Whereas leaf assimilation of substrate CO_2 (A_n) declined, consumption of substrate CHO in roots (R_r) increased, in both cotton (Fig. 6A) and melon (Fig. 6B). The greatest impact in both species was observed between the CF and MO3 treatments, with little further increase between MO3 and HO3.

The species differences in physiological activity observed in gas exchange parameters under CF conditions (1.5-fold greater A_n in melon than cotton; cf. Figs 2A, 3A) were reflected in CF values of R_r (1.7-fold greater in melon than cotton; cf. Fig. 6A, B). However, in contrast to the greater sensitivity of gas exchange in melon, the sensitivity of R_r was similar in the two species. R_r increased by about 1.2-fold in MO3 and only slightly more in HO3. Thus, O_3 caused an increase in R_r of both species, by contrast with the decrease in A_n.

Acute responses of fine root respiration (R_r) to short-term exposures to O_3 were difficult to resolve. Following 1 h of exposure to O_3, R_r of cotton was increased by about 15% (P=0.056; Fig. 7A). Measurements were consistently greater in HO3 than in CF-treated roots of cotton, but the differences at individual timepoints were not generally statistically significant. Over all measurements R_r of cotton was significantly increased by the HO3 treatment (P=0.029). There was no diurnal pattern in values of R_r, consistent with the observations of Walters *et al.* (1993) in a variety of temperate tree species.

Short-term responses of R_r to O_3 in melon (Fig. 7B) were less clear than those in cotton. At three out of five measurement timepoints, R_r was increased by O_3 exposure. At the first (1 h) observation, enhancement of R_r was similar to that observed in cotton (about 15%) though not statistically significant due to considerable variability. At the last measurement timepoint (53 h) the enhancement of R_r was significant (P=0.043), but attributed largely to the unexplained decline in mean CF values. Over all measurements in melon, the experiment did not resolve a significant effect of O_3 on R_r.

The rates of R_r observed in both species are consistent with many similar measurements (Reich *et al.*, 1998;

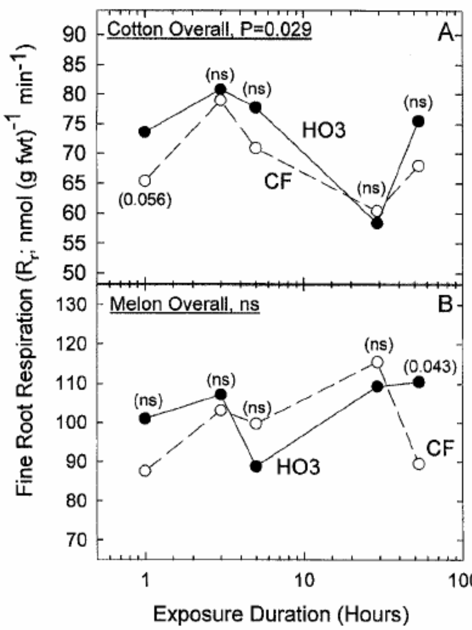

Fig. 7. Effect of acute (short-term) exposure to contrasting O_3 concentrations on respiration of fine roots on a fresh weight basis (A, cotton; B, melon). Mean ±SE. Note log scale for time axis.

Edwards, 1991; Gunn and Farrar, 1999; Singh and Blanke, 2000). These rates, measured in the terminal 3–4 cm of the root system, are representative of the whole fine root system (i.e. most of the roots in these young experimental plants) as suggested by the longitudinal mapping of R_r along roots of peach (Bidel *et al.*, 2001).

The difficulty in resolving acute impacts of O_3 on R_r was further explored through a test of the respirometry methodology. Responses of R_r in cotton were contrasted following exposure to dark or sunlight. By contrast with the variable and modest increase in R_r following O_3 exposure (Figs 6A, 7A), darkness clearly suppressed R_r (P=0.04; Table 3), using the same techniques with the same species and a much smaller sample size (n=12 plants).

This reduction in R_r was expected as a consequence of short-term reduction in export of CHO to the root system in darkened plants, and resulting substrate limitation of R_r (Begna *et al.*, 2002; Dwivedi, 2000; Lambers *et al.*, 1996; Walters *et al.*, 1993). The contrast between these results and the opposing changes in A_n and R_r following O_3-exposure suggests that linkages other than substrate availability may operate at the whole plant level to mediate O_3 phytotoxicity. For example, effects of altered irradiance on A_n may precede effects on R_r (e.g. in *Lolium multiflorum*; Hansen and Jensen, 1977), suggesting a lag due to transport and temporal buffering due to utilization of stored CHO in sink respiration. In the present studies no consistent lag was observed between O_3 impacts on A_n and

Table 3. *Effect of dark adaptation on fine root respiration of cotton* (n=*12*; *mean* ±*SE)*

	Respiration (nmol O_2 g^{-1} FW s^{-1})	Significant difference
Sunlit	128±12	P=0.040
Dark	96±8	

on R_r, and the effects were in opposing directions. The hypothesized impact of substrate limitation caused by O_3 was not observed. No direct role of limitation of R_r by substrate CHO is suggested.

The acute, short-term responses of R_r are consistent with the conclusions from the longer term, chronic exposure experiments. This is particularly so in cotton, in which significant differences were observed within individual time-points and experiment-wise comparisons. These results are also supported by those of Scagel and Andersen (1997), who found consistent, though generally non-significant, increases in fine root respiration in Ponderosa pine growing in both high and low fertility media, particularly late in the growing season. Kelting *et al.* (1995) also observed a 50% enhancement of fine root respiration in O_3-exposed red oak. These effects derived from opposing non-significant effects of O_3 on respiration and biomass of roots. The current results are also consistent with the unpublished observations with rooted single leaves of Pima cotton (S Gunn and DA Grantz, unpublished data), in which acute exposure to O_3 (as described in Grantz and Farrar, 1999, 2000) consistently, but non-significantly, increased R_r.

Coupling of source strength and sink respiration

The quantity of carbohydrate (CHO) available for export from source leaves to sink tissues such as fine roots is reduced by the direct and indirect impacts of O_3 on A_n, as well as by O_3-inhibited export of recent photosynthate from source leaves (Grantz and Farrar, 1999, 2000; Darrall, 1989). This suggests that O_3 could reduce R_r in response to reduced substrate availability.

O_3 has been observed to reduce R_r in some cases (Edwards, 1991; Hofstra *et al.*, 1981; Ito *et al.*, 1985; Gorisson and van Veen, 1988; Shan *et al.*, 1996). This might be attributed to reduced import of current photosynthate into respiring fine roots and resultant substrate limitation of R_r. Clearly, darkening cotton plants in the present study (Table 3) resulted in the rapid down-regulation of fine root respiration. Reich *et al.* (1998) found a strong correlation between A_n and R_r in a number of northern tree species, particularly when A_n was expressed on a leaf mass basis. However, the addition of exogenous sucrose to roots did not reliably increase R_r in several grass species (Gunn and Farrar, 1999), suggesting that R_r does not operate at the margin of substrate availability, except perhaps following a reduction in irradiance (Begna *et al.*, 2002).

In the present studies, A_n, a primary measure of current photosynthate available for export from source leaves to sink tissues such as fine roots, was significantly reduced by O_3 at 5 h and 29 h of exposure in cotton and at 3, 5, and 53 h in melon. It is clear that neither the time-course nor magnitude of O_3-induced reduction of current photosynthate (A_n) is correlated with any similar reduction in R_r in either cotton or melon. Indeed, regression analysis (not shown) demonstrates a pronounced (non-significant) negative trend between A_n and R_r in both cotton and melon. The negative relationships describe both the control and O_3-treated plants. Inhibition of A_n and the potential reduction in CHO available to roots does not appear to control R_r over the initial period of exposure to O_3 (1–53 h) nor over the longer time scales of the chronic exposure experiments (5–6 weeks).

No mechanistic explanation is yet available for these opposing trends in A_n and R_r (i.e. in the present experiments and those of Scagel and Andersen, 1997; Kelting *et al.*, 1995). The simultaneous occurrence of increased R_r and inhibited root growth in all these studies suggests that O_3 impacts on below-ground carbon storage and root system development could reflect diversion of CHO to R_r at the expense of root growth. However, this conclusion raises the question of the initial mechanism of O_3-enhanced root respiratory activity.

The lack of positive correlation between O_3 effects on A_n and R_r suggests a non-substrate linkage between these two carbon fluxes, both of which are clearly affected by O_3. Following O_3 exposure, shoot-sourced phytohormones could signal leaf demand for nutrients to support foliar repair processes (Kelting *et al.*, 1995), potentially stimulating accelerated uptake of minerals for export to the foliage in xylem fluid. Similarly, the depletion of nutrients from the root tissues, reflecting enhanced utilization in the shoot, could stimulate nutrient uptake by roots. Both would increase R_r (Singh and Blanke, 2000). An analogous response was observed following chronic (6 week) deficiency of K^+ in *Brassica oleracea* (Singh and Blanke, 2000), in which R_r increased as root growth declined. However, in the case of K^+ deficiency the root:shoot biomass ratio increased, in contrast to the reduction observed following exposure to O_3 (Grantz and Yang, 1996).

Increased R_r could reflect damage to root tissues associated with O_3 exposure of the shoot. No mechanism for such destructive linkage has been suggested. Nevertheless, a potential toxic response is suggested in at least one species (*Picea abies*) by observations of cytological damage in root meristems (Muller and Grill, 1994; Muller *et al.*, 1994*a*, *b*). The respiratory quotient (Q) may also reflect tissue damage. Scagel and Andersen (1997) observed an increase in R_r following O_3 exposure in Ponderosa pine, accompanied by an increase in (Q). Carbon starvation of roots of *Zea mays* led to proteolysis, release of N, and increases of specific amino acids, particularly asparagine following dark-treatment (Brouquisse *et al.*, 1992, 1998), indicative of metabolic derangement. The extent to which this occurs following O_3 exposure is unknown.

Alternatively, the increase in Q may indicate respiratory utilization of increasingly complex CHO substrates following O_3 exposure. It is noteworthy that in the present experiments R_r increased following O_3 exposure in both the sucrose-transporting cotton and the predominant stachyose-transporting melon. Changes in the sugars involved in translocation from source leaves to respiring roots following exposure to O_3 have not been adequately addressed. Preliminary indications suggest that O_3 exposure shifts the soluble sugar pool in fine roots, by reducing the relative concentration of sucrose and increasing that of stachyose, in both cotton and melon (DA Grantz, unpublished observations). More mechanistic investigation of potential O_3 impacts on phloem loading and the resulting profiles of CHO in the phloem sap and in sink tissues may warrant further investigation.

Conclusions

Chronic exposure to O_3 reduces carbon assimilation while increasing respiration in fine roots. Acute responses of plants transferred to O_3-containing environments demonstrate that these physiological responses occur within hours, and roughly in parallel in leaf and shoot. As these carbon fluxes (A_n and R_r) are in opposing directions, a simple substrate linkage is unlikely. Reduced root growth and O_3-induced reductions in root:shoot biomass ratio are consistent with O_3-limitation of carbohydrate transport to developing roots. The increased respiratory activity may reflect linkage between shoot and root involving demand for nutrients, although this generally results in increased root:shoot ratio. Alternatively, the linkage may reflect changes in the sugar profiles available to support root respiration, possibly attributed to changes in phloem loading in source leaves. Yet another alternative is the transfer of phytotoxic materials from O_3-damaged leaves to roots, inducing damage and enhancing R_r associated with repair mechanisms in roots. At present data are insufficient to resolve these possibilities.

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